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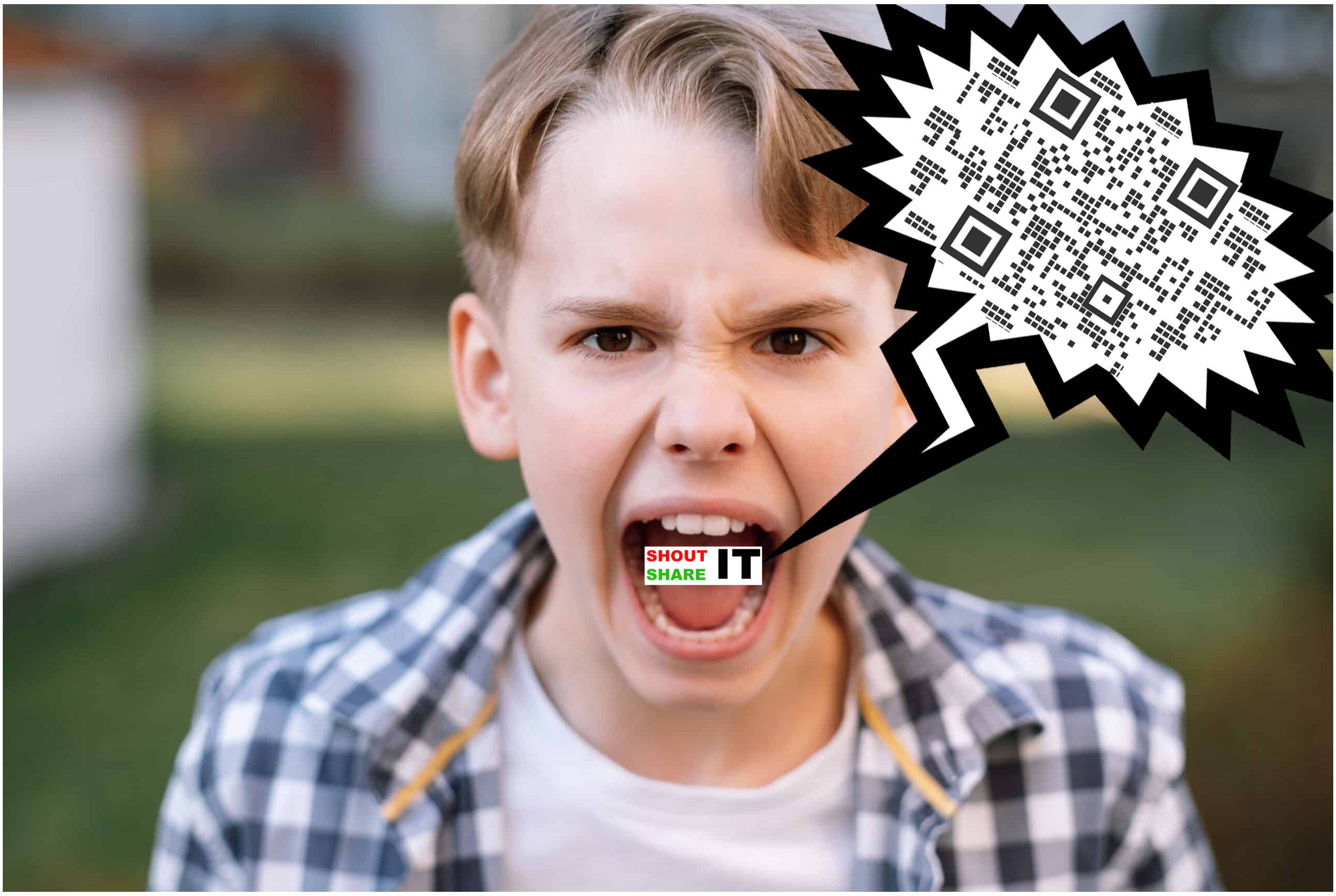

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The Organisms Living Around Energized Submarine Power Cables, Pipe, and Natural Sea Floor in the Inshore Waters of Southern California

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Abstract.—Between 1 February 2012 and 26 February 2014 using scuba, we surveyed the fishes, invertebrates, and macrophytes living on two energized submarine power cables, an adjacent pipe, and nearby natural habitat in southern California at bottom depths of 10–11 m and 13–14 m. Over the course of the study, average electromagnetic field (EMF) levels at the two cables (A and B) were statistically similar (Cable A = 73.0μT, Cable B = 91.4μT) and were much higher at these two cables than at either the pipe (average = 0.5μT) or sand (0μT). Overall, our study demonstrated that 1) the fish and invertebrate communities on cables, pipe, and natural habitat strongly overlapped and 2) there were no differences between the shallower and deeper fish and invertebrate communities. We saw no evidence that fishes or invertebrates are either preferentially attracted to, or repelled by, the EMF emitted by the cables. Any differences in the fish or invertebrate densities between cables, pipe, and natural habitat taxa were most likely due to the differences in the physical characteristics of these habitats. As with the fishes and invertebrates, macrophytes did not appear to be responding to the EMF emitted by the cables. Rather, it is likely that differences in the plant communities were driven by site depth and habitat type.

It is likely that for the foreseeable future, offshore renewable energy technologies will focus on the generation of electricity from renewable resources (e.g., wind and wave). Specifically in U.S. waters, there has been substantial interest in wind energy off the East Coast of the United States (Petruny-Parker et al. 2015; BOEM 2014), both wind and wave energy off the Pacific Coast (Boehlert et al. 2013), and harnessing tidal energy in Puget Sound (Thomson et al. 2012). These technologies harness energy from an array of individual devices and, through power cables, send electricity to shore via cables. These cables will transmit either alternating current or direct current, and, if the cable uses alternating current, this current will generate both electric and magnetic fields around these cables.

Research has shown that cartilaginous and some bony fishes, as well as at least some invertebrates, are sensitive to electromagnetic fields (EMF) and that these fields can alter the behavior of these organisms (Kalmijn 1982; Formicki et al. 2004; Tanski et al. 2005; and summarized in Normandeu et al. 2011). However, worldwide, only a few studies have been conducted to document the effects of EMF on marine organisms *in situ* (DONG Energy and Vattenfall 2006; Ohman et al. 2007; Westerberg and Lagenfelt 2008) or in a semi-artificially enclosed mesocosm (Gill et al. 2012). These studies have yielded either equivocal, or at best subtle, evidence of marine organisms responding to artificially induced EMF in a natural or semi-natural environment.

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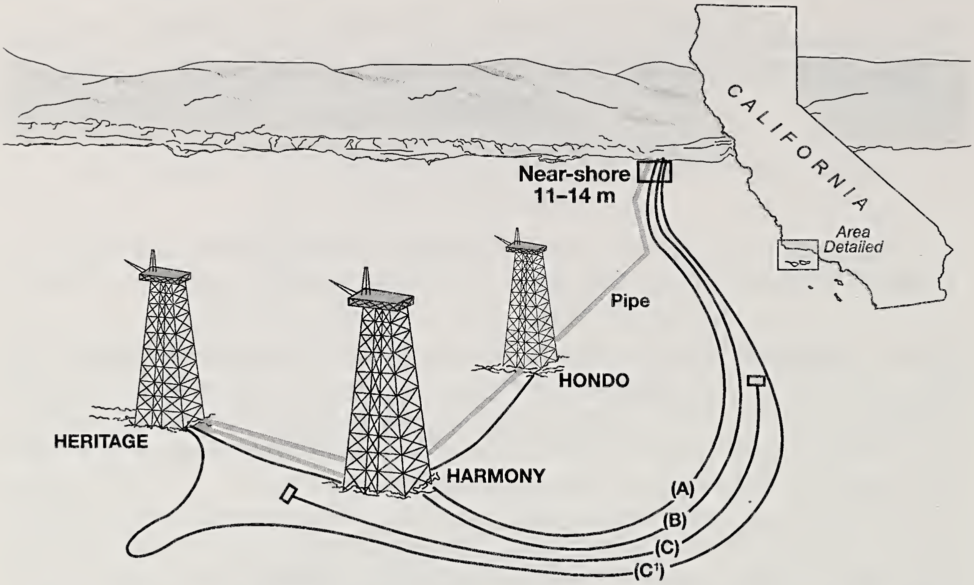

Fig. 1. Location of the energized and unenergized submarine power cables and pipe in the study area.

Submarine transmission cables that power offshore oil platforms in the Pacific Region provide an opportunity to examine potential responses of marine organisms to offshore renewable energy development (Fig. 1). We note that these power cables are industry standard, the type that will be used for connecting devices (35 KV) within renewable energy installations.

Specific objectives of this study were to determine:

1) If differences exist among fish, invertebrate, and plant communities associated with an energized cable habitat and those communities around a nearby pipe and soft seafloor lacking an energized cable.
2) If electro-sensitive species that are regionally important, such as sharks and rays, respond (via either attraction or repulsion) to the EMFs of an *in situ* power transmission cable.
3) The potential effectiveness of the commonly proposed mitigation of cable burial.

Materials and Methods

Our surveys were conducted by scuba off the coast of Las Flores Canyon, southern California (34°27.6’N, 120°02.7’W). In this area there are 1) three 20.32 cm (8”) diameter submarine power cables (variously energized and unenergized) providing power to three offshore oil platforms and 2) a 30.48 cm (12”) diameter pipe running from the platforms to shore (Fig. 1). The furthest distance between the outermost cable and the pipe is about 40 m.

Prior to beginning the study, we found that sections of cable were both exposed and buried by natural disturbances and that EMF levels were lower on the sandy substrate directly over the buried cable than on the exposed cable. Thus to study the effect of the maximum EMF possible, we determined the survey would be conducted along unburied sections of the cable. Divers observed cables and pipeline for exposed continuous 30-m long sections, a standard transect length that we and other research groups have used for fish surveys in the region. We were able to find sufficient lengths of exposed energized cables (known as cables A and B) where fixed

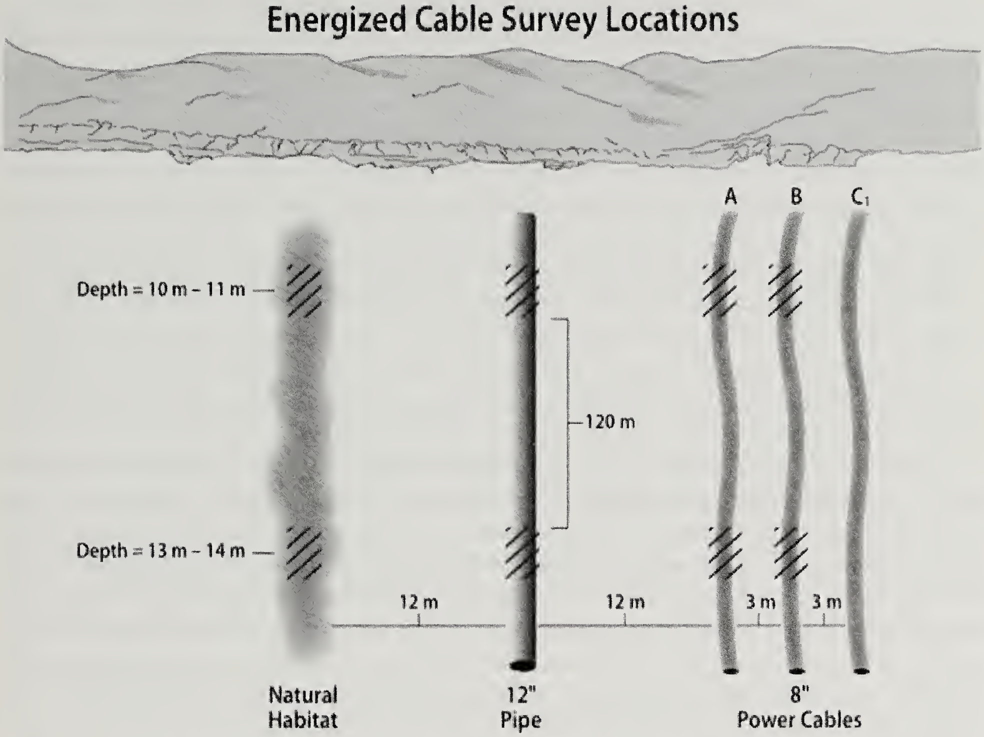

Fig. 2. Schematic illustration of cables, pipe, and natural habitat surveyed by scuba, 1 February 2012–26 February 2014. Cables A and B were energized and were used in this study. The nearshore section of Cable C has been removed. Cable C^1 was unenergized and was not used in this study as it was mostly buried in the sea floor. Distance between cables, pipe, and natural habitat not drawn to scale.

30-m long transects could be set at two bottom depths. An unenergized cable (known as cable C1) was mostly buried, and we did not find any exposed 30-m lengths. Thus, for these surveys, we used the nearby exposed pipe as a surrogate for an unenergized cable. Divers surveyed fishes, invertebrates, and macrophytes along three habitats: an energized submarine power cable, a pipe, and a sandy, natural control area to the west of both cables and pipe (Fig. 2).

The experimental design was comprised of six fixed 30 m-long transects (treatments) of which one was installed in each of three habitats (along a cable, the pipe, and over sandy bottom) and in each of two depths (shallow, 10–11 m; and slightly deeper waters, 13–14 m depth). The end of the shallow transects and beginnings of the deep ones were separated by about 120 m. The beginning and ending of each transect in each habitat was marked by sand anchors as was each 5 m segment along each transect. Transects were 2 m wide, centered on the pipe or cable or an imaginary line between sand anchors that delineated the sandy control transect. Surveys of fishes, macroinvertebrates, and macrophytes were conducted every 2–4 weeks along the six transects during daylight hours by two divers.

At the beginning of the study we measured the EMF emitted by the power cables in our study area and found that two cables, A and B, were energized. We began our cable surveys on energized cable B. However, on 15 May 2013, we detected that cable B had become unenergized and we switched our surveys to energized cable A for the duration of the study. Importantly, both cables A and B had been energized for at least several years before cable B was switched off (D. Gilbert, pers. comm. to M. L.). The magnitude of EMF was measured at the beginning of each survey. A diver recorded readings from a detector placed directly against the cable, pipe, and sand. We used the Kruskal-Wallis one-way ANOVA on ranks to test for a difference in EMF field strength among habitats (Cable B, Cable A, Pipe, and natural habitat). The Wilcoxon test was used for nonparametric comparisons to identify where the EMF differed. The first diver surveyed all fishes encountered within 2 m above the substrate. Fish were identified to species, counted, and sized by eye to the nearest centimeter. A second diver followed and recorded the number of macrophytes in the 2 m swath centered over the cable and pipe or on the sand. The quantification of macrophytes was used to determine if these structure-forming organisms differentially modified the habitats. The second diver also recorded macroinvertebrates (i.e., cnidarians, mollusca, crustaceans, and echinoderms) encountered within the same 2-m-wide sampling area. Only individual invertebrates of at least 10 cm in any dimension were recorded.

We used the Kolmogorov Smirnov Two-Sample test to evaluate whether the size frequency distribution of all fishes differed between the cable, pipe, and natural habitats. Mean lengths among habitats were compared using Welch's test. The fish length observations from both cables A and B were combined for this analysis. We employed the permutational analysis of variance routine in PERMANOVA + for PRIMER (Anderson et al. 2008) to test the response (counts per transect) of the fish, invertebrate, and macrophyte communities, separately, to one or more of the factors: habitat (cable, pipe, natural), depth (shallow, deep), and time (survey). The dataset was divided into two periods; the first when cable B was surveyed and the second when cable A was surveyed. This was a reasonable approach given that the two cable environments were seen to be quite different in terms of the structure-forming macrophyte community that could possibly affect fish and mobile macroinvertebrate abundance. By analyzing data separately from each cable, we were able to determine whether, if a species was more abundant at either a cable or pipe, this pattern occurred at both energized cables (implying that EMF may have been responsible) or only at one of the cables (implying that some other environmental factor was responsible). The experimental design was a balanced, repeated measures 3-way analysis with fixed factors for each period of surveys. During each survey date, we sampled six different treatments (i.e., transects) without replication. The terms of the model were habitat, depth, time, habitat x depth, habitat x time, and depth x time. If the effect of habitat on the abundance of a species was significant ($p<0.05$), then posthoc PERMANOVA permutational t-tests were run for independent pairwise comparisons of abundance between cable, pipe, and natural habitat. P-values in the PERMANOVA routines were calculated using 9999 permutations that generated the test statistic distribution under a true null hypothesis based on the resemblance between the samples. The observations of fish and invertebrate counts per transect were log $(x+1)$ transformed and the macrophyte dataset of counts per transect was square-root transformed before calculating the Bray-Curtis similarity coefficients that quantified the resemblance between multivariate transect samples. We used the PERMDISP routine in PRIMER to determine that either $\log(X+1)$ or square root transformations of the abundance data (count per transect) reduced the heterogeneity of dispersion of multivariate samples among the different experimental treatments. Similarly, we used the permutational analysis of variance routine to test the response (number per transect) of abundant individual species of fishes, invertebrates, and macrophytes to the same factors. The individual species data were transformed using either $\log(x+1)$ or square-root of x. The test statistic for individual species is based on a Euclidean distance matrix between samples (Anderson et al. 2008).

We used multidimensional scaling (MDS) ordination in PRIMER v6 (Clarke and Gorley, 2006) to visualize assemblage groupings of transect samples by habitat and depth. As a complement to the PERMANOVA analysis, we used a two-way crossed analysis of similarity (ANOSIM) in PRIMER to evaluate the degree of overlap in species composition across habitat and depth.

Table 1. All dates of surveys on energized cables, pipe, and natural habitat. Fishes and plants were surveyed on all dates; invertebrates were surveyed from 22 June 2012 to 26 February 2014. Surveys were conducted on energized Cable B from 1 February 2012 to 15 May 2013 and on energized Cable A from 14 June 2013 to 26 February 2014.

2012								
1 Feb.	22 Feb.	8 Mar.	27 Mar	12 Apr.	24 Apr.	9 May	8 June	22 June
13 July	25 July	10 Aug.	22 Aug	11 Sept.	2 Nov.	7 Dec.		
2013								
8 Jan.	5 Feb.	28 Feb.	12 March	3 Apr.	24 Apr.	3 May	15 May	14 June
9 July	16 Aug.	30 Aug.	13 Sept.	30 Sept.	18 Oct.	8 Nov.	20 Nov.	6 Dec.
31 Dec.								
2014								
15 Jan.	12 Feb.	26 Feb.						

ANOSIM operates on the resemblance matrix to test the null hypothesis that there are no assemblage differences between pipe, cable, and natural habitats (factor A), allowing that there may be shallower/deeper differences (factor B), The ANOSIM sample test statistic, R, ranges from 0 (no difference between groups) to 1 (all dissimilarities between the groups are larger than any dissimilarities among samples with either group). A statistically significant ($p<0.05$) but negligibly small R-value close to 0 indicates that species composition differ between habitats, but strongly overlap.

Results

From 1 February 2012 to 26 February 2014, fishes and macrophyte surveys were conducted from the beginning to end of the study. Invertebrate surveys were conducted beginning on 22 June 2012 and continued until the end of the study. Surveys were conducted on a total of 38 days (Table 1). We note that the natural habitat was sand and eelgrass. Over the course of the study, average EMF levels at the two cables (A and B) were statistically similar (Cable A = 73.0μT, Cable B = 91.4μT) and were statistically higher at the two cables compared to the pipe (average = 0.5μT) or sand (0μT) (Fig. 3, Table 2). We note that previous studies have demonstrated that EMF levels reach background levels about one meter from this cable (Love et al. 2015, 2016).

We found that the fish community varied among the cables, pipe, and natural habitat; however, there was significant interaction between the effects of habitat and depth on assemblage structure (Table 3). Furthermore, the 3-dimensional MDS plots of the assemblages from transect samples demonstrates there is substantial overlap of the habitat groupings during the periods when Cable A (global R=0.043, p=0.007; Fig. 4) and Cable B were surveyed (global R=0.253, p=0.0001; Fig. 5). Depth-related differences appeared to be somewhat more pronounced when

Table 2. Wilcoxon test values comparing EMF field strengths of two energized cables, pipe, and natural habitat, 2012–2014. NH = natural habitat.

Site	Site	Mean Difference	Standard Error	Z	p-value
Cable B	Cable A	5.95	4.15	1.43	0.15
NH	Cable A	−32.46	4.40	−7.38	<.0001
Pipe	Cable A	−34.46	5.30	−6.50	<.0001
Pipe	Cable B	−36.39	5.30	−6.87	<.0001
NH	Cable B	−36.97	4.67	−7.92	<.0001
NH	Pipe	−43.74	5.34	−8.18	<.0001

Table 3. P-values from the repeated measures PERMANOVA testing the effects of habitat (HA), depth (IN), and sampling date (DA) on fish community structure and density of individual taxa and from PERMANOVA pairwise tests for differences between habitats for the periods when Cables B and A were surveyed. YOY = young-of-the-year. Species selected were those that comprised at least 1% of all fishes observed. Statistically significant results are underlined.

Cable B	HA	IN	DA	HAxIN	HAxDA	INxDA	Cable, pipe	Cable, natural	Pipe, natural
Fish community	0.0001	0.0287	0.0001	0.0127	0.5245	0.1356	0.0016	0.0051	0.0002
Taxon:									
Oxyjulis californica	0.0347	0.9836	0.0001	0.0144	0.8534	0.7389	0.0470	0.0546	0.9695
Citharichthys spp.	0.0007	0.4798	0.0007	0.0325	0.8485	0.0311	0.0001	0.1107	0.0494
Phanerodon furcatus	0.1716	0.1071	0.0313	0.1453	0.7047	0.9496			
Cymatogaster aggregata	0.1005	0.6411	0.0001	0.8181	0.0014	0.9112			
KGB YOY[1]	0.0038	0.0341	0.0006	0.2227	0.1552	0.0718	0.5834	0.0008	0.0092
Brachyistius frenatus	0.3762	0.1600	0.4938	0.4066	0.5033	0.5837			
Sebastes miniatus YOY	0.0916	0.0001	0.0001	0.0121	0.1177	0.0001			
Embiotoca jacksoni	0.0033	0.0916	0.0162	0.8015	0.0412	0.3053	0.0473	0.0661	0.0077
Aulorhynchus flavidus	0.4649	0.3638	0.4685	0.1924	0.4316	0.4712			
Hypsurus caryi	0.4824	0.0381	0.0037	0.1316	0.7651	0.1409			
Damalichthys vacca	0.0573	0.1179	0.4520	0.0224	0.4984	0.4779			
Sebastes caurinus	0.4054	0.3437	0.3529	0.4371	0.5111	0.4941			
Sebastes paucispinis YOY	0.1283	0.4334	0.0667	0.6773	0.9587	0.1426			
Sebastes serranoides/Sebastes flavidus YOY	0.3805	0.2238	0.0121	0.9045	0.8630	0.0053			
Heterostichus rostratus	0.0318	0.2473	0.0162	0.0148	0.6152	0.9351	0.5088	0.0085	0.0092

Table 3. Continued.

Cable A	HA	IN	DA	HAxIN	HAxDA	INxDA	Cable, pipe	Cable, natural	Pipe, natural
Fish community	0.0001	0.0001	0.0001	0.0002	0.0085	0.0001	0.0004	0.0003	0.0001
Taxon:									
Oxyjulis californica	0.0027	0.0161	0.0001	0.0356	0.5450	0.0002	0.0210	0.0949	0.0105
Citharichthys spp.	0.0022	0.0001	0.1479	0.1831	0.5405	0.3673	0.0068	0.8271	0.0025
Phanerodon furcatus	0.0199	0.1778	0.0126	0.2533	0.1704	0.0041	0.0516	0.0108	0.2880
Shiner perch	0.0406	0.5422	0.0291	0.6874	0.0094	0.4952	0.3513	0.0165	0.1574
KGB YOY[1]	0.0001	0.0067	0.0001	0.0466	0.0028	0.1993	0.0001	0.0002	0.0001
Brachyistius frenatus	0.0166	0.0096	0.2912	0.0111	0.5226	0.2729	0.0409	0.0344	0.3601
Sebastes miniatus YOY	0.3610	0.0793	0.0660	0.0805	0.1797	0.0528			
Embiotoca jacksoni	0.0001	0.0001	0.0062	0.0002	0.4926	0.0375	0.1134	0.0003	0.0001
Aulorhynchus flavidus	0.4554	0.7378	0.3142	0.8891	0.9822	0.8205			
Hypsurus caryi	0.0004	0.0006	0.0314	0.0093	0.8955	0.2189	0.0376	0.0429	0.0001
Damalichthys vacca	0.0691	0.0012	0.4786	0.3506	0.4163	0.2530			
Sebastes caurinus	0.0004	0.2144	0.0008	0.3915	0.0298	0.3777	0.1484	0.0003	0.0020
Sebastes paucispinis YOY	0.0438	0.8801	0.8572	0.7147	0.7701	0.5658	0.0480	0.1252	0.3454
Sebastes serranoides/Sebastes flavidus YOY	0.4668	0.9484	0.1391	0.3712	0.9601	0.7776			
Heterostichus rostratus	0.0050	0.0306	0.1265	0.0145	0.1581	0.0474	0.0311	0.0070	0.2236

[1] *Sebastes atrovirens, S. caurinus, S. carnatus, S. chrysomelas*

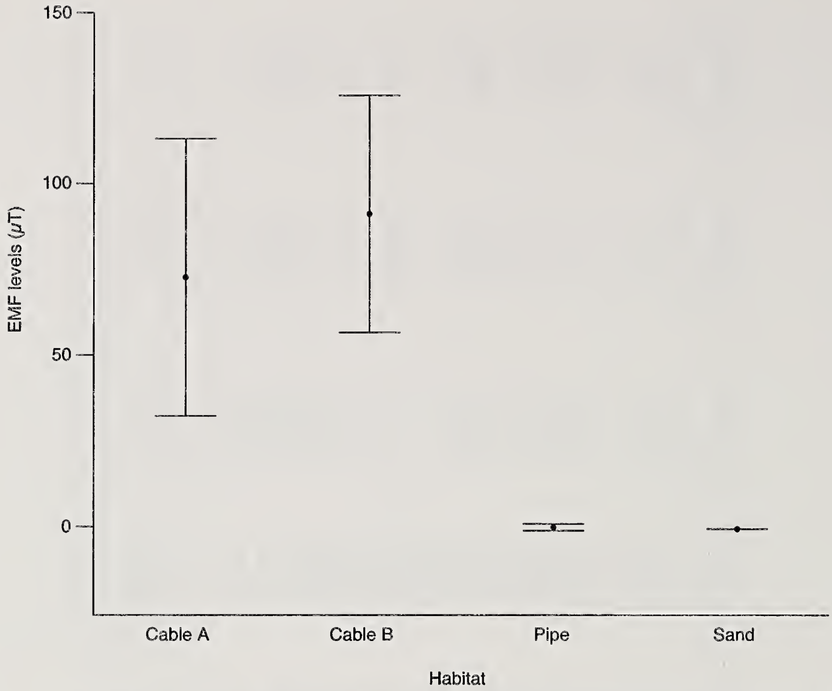

Fig. 3. Electromagnetic field levels measured on cables, pipe, and natural habitat surveyed from 1 February 2012–26 February 2014. Vertical bars represent standard errors.

Cable A is compared to the pipe and natural habitat rather than in similar comparisons with Cable B (Fig. 4 and Fig 5, respectively).

We conducted a total of 38 days of fish surveys during three years. Over all habitats, 4465 individuals representing at least 44 species (summed from Tables 4, 5) were observed. Dominant species included adults of benthic-oriented, schooling taxa (i.e., *Oxyjulis californica, Brachy-*

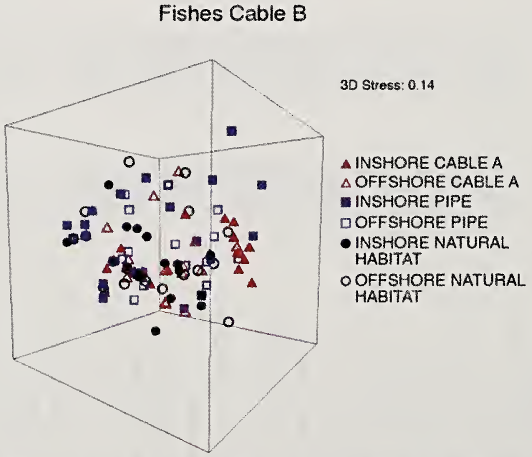

Fig. 4. A 3-d multiple dimensional scaling model comparing the fish assemblages from shallower and deeper transects in Cable B, pipe, and natural habitat.

Table 4. Total count across all habitats and mean (SE) number of fishes per transect in each habitat during period when Cable B was surveyed, 1 February 2012–15 May 2013. Two transects, shallow and deep, were surveyed in each habitat on 23 sampling dates. Number of transect surveys in each habitat, n = 46. YOY = young-of-the-year.

	All habitats	Cable B		Pipe		Natural	
Scientific name	Count	Mean	(SE)	Mean	(SE)	Mean	(SE)
Oxyjulis californica	460	5.35	(1.75)	2.78	(1.63)	1.87	(0.95)
Citharichthys spp.	496	5.48	(1.19)	1.87	(0.48)	3.43	(0.69)
Phanerodon furcatus	111	0.78	(0.48)	0.20	(0.09)	1.43	(0.83)
Cymatogaster aggregata	278	1.13	(0.69)	0.09	(0.06)	4.83	(4.35)
Sebastes atrovirens, S. caurinus, S. carnatus, or *S. chrysomelas* YOY	180	1.72	(0.58)	2.11	(0.83)	0.09	(0.04)
Brachyistius frenatus	75	0.39	(0.15)	1.22	(1.02)	0.02	(0.02)
Sebastes miniatus YOY	228	1.13	(0.57)	3.30	(1.85)	0.52	(0.30)
Sebastes jordani YOY	190	0.43	(0.43)	1.09	(1.09)	2.61	(2.61)
Embiotoca jacksoni	78	0.39	(0.13)	1.20	(0.46)	0.11	(0.09)
Aulorhynchus flavidus	158	0.72	(0.67)	0.41	(0.18)	2.30	(1.82)
Hypsurus caryi	26	0.15	(0.07)	0.28	(0.12)	0.13	(0.07)
Damalichthys vacca	119	0.02	(0.02)	2.54	(1.94)	0.02	(0.02)
Sebastes caurinus	52	0.74	(0.41)	0.33	(0.14)	0.07	(0.04)
Sebastes paucispinis	81	0.28	(0.20)	1.46	(0.88)	0.02	(0.02)
Sebastes serranoides or *S. flavidus* YOY	40	0.13	(0.08)	0.67	(0.57)	0.07	(0.04)
Heterostichus rostratus	23	0.30	(0.15)	0.20	(0.11)	0.00	(0.00)
Sebastes semicinctus	29	0.22	(0.18)	0.41	(0.39)	0.00	(0.00)
Synodus lucioceps	1	0.00	(0.00)	0.00	(0.00)	0.02	(0.02)
Oxylebius pictus	12	0.09	(0.04)	0.17	(0.06)	0.00	(0.00)
Sebastes mystinus	1	0.00	(0.00)	0.02	(0.02)	0.00	(0.00)
Sebastes auriculatus	17	0.00	(0.00)	0.37	(0.35)	0.00	(0.00)
Scorpaenichthys marmoratus	7	0.07	(0.04)	0.09	(0.04)	0.00	(0.00)
Hexagrammos decagrammus	2	0.00	(0.00)	0.04	(0.03)	0.00	(0.00)
Pleuronichthys coenosus	6	0.04	(0.03)	0.02	(0.02)	0.07	(0.05)
Gibbonsia spp.	2	0.02	(0.02)	0.02	(0.02)	0.00	(0.00)
Sebastes dalli	5	0.04	(0.03)	0.07	(0.07)	0.00	(0.00)
Paralabrax clathratus	1	0.02	(0.02)	0.00	(0.00)	0.00	(0.00)
Unidentified Cottidae	3	0.00	(0.00)	0.07	(0.04)	0.00	(0.00)
Leiocottus hirundo	4	0.00	(0.00)	0.07	(0.05)	0.02	(0.02)
Neoclinus blanchardi	1	0.02	(0.02)	0.00	(0.00)	0.00	(0.00)
Ophiodon elongatus	5	0.04	(0.03)	0.00	(0.00)	0.07	(0.05)
Paralichthys californicus	1	0.00	(0.00)	0.00	(0.00)	0.02	(0.02)
Sebastes atrovirens	3	0.02	(0.02)	0.04	(0.03)	0.00	(0.00)
Unidentified fishes	3	0.07	(0.05)	0.00	(0.00)	0.00	(0.00)
Gibbonsia spp.	2	0.00	(0.00)	0.04	(0.03)	0.00	(0.00)
Sebastes carnatus	1	0.02	(0.02)	0.00	(0.00)	0.00	(0.00)
Paralabrax nebulifer	1	0.02	(0.02)	0.00	(0.00)	0.00	(0.00)
Pleuronichthys decurrens	1	0.00	(0.00)	0.00	(0.00)	0.02	(0.02)
Porichthys spp.	1	0.00	(0.00)	0.02	(0.02)	0.00	(0.00)
Rathbunella spp.	1	0.00	(0.00)	0.02	(0.02)	0.00	(0.00)
Sebastes rastrelliger	1	0.00	(0.00)	0.02	(0.02)	0.00	(0.00)
Urobatis halleri	1	0.02	(0.02)	0.00	(0.00)	0.00	(0.00)
All fishes	2707	19.93	(3.91)	22.24	(5.56)	17.76	(5.85)

Table 5. Total count across all habitats and mean (SE) number of fishes per transect in each habitat during period when Cable A was surveyed, 14 June 2013–26 February 2014. Two transects, shallow and deep, were surveyed in each habitat on 14 sampling dates. Number of transect surveys in each habitat, n = 28. YOY = young-of-the-year.

	All habitats	Cable A		Pipe		Natural	
Scientific name	Count	Mean	(SE)	Mean	(SE)	Mean	(SE)
Oxyjulis californica	414	3.96	(1.14)	8.46	(2.13)	2.36	(0.56)
Citharichthys spp.	140	2.14	(0.50)	0.71	(0.20)	2.14	(0.48)
Phanerodon furcatus	241	4.29	(1.29)	2.75	(1.19)	1.57	(0.65)
Cymatogaster aggregata	56	0.00	(0.00)	0.29	(0.29)	1.71	(1.43)
Sebastes atrovirens, S. caurinus, S. carnatus, or *S. chrysomelas* YOY	151	1.79	(0.54)	3.50	(0.81)	0.11	(0.06)
Brachyistius frenatus	199	6.18	(2.76)	0.79	(0.64)	0.14	(0.11)
Sebastes miniatus YOY	27	0.36	(0.15)	0.43	(0.17)	0.18	(0.15)
Sebastes jordani YOY	0	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)
Embiotoca jacksoni	104	1.68	(0.70)	2.00	(0.48)	0.04	(0.04)
Aulorhynchus flavidus	7	0.07	(0.05)	0.04	(0.04)	0.14	(0.07)
Hypsurus caryi	123	1.29	(0.30)	2.61	(0.60)	0.50	(0.23)
Damalichthys vacca	23	0.29	(0.15)	0.46	(0.17)	0.07	(0.05)
Sebastes caurinus	54	0.71	(0.27)	1.21	(0.42)	0.00	(0.00)
Sebastes paucispinis	18	0.54	(0.24)	0.00	(0.00)	0.11	(0.11)
Sebastes serranoides or *Sebastes flavidus* YOY	32	0.43	(0.14)	0.43	(0.26)	0.29	(0.25)
Heterostichus rostratus	22	0.54	(0.18)	0.18	(0.07)	0.07	(0.05)
Sebastes semicinctus	15	0.21	(0.15)	0.32	(0.25)	0.00	(0.00)
Synodus lucioceps	26	0.79	(0.68)	0.00	(0.00)	0.14	(0.14)
Oxylebius pictus	13	0.29	(0.13)	0.18	(0.09)	0.00	(0.00)
Sebastes mystinus	19	0.36	(0.19)	0.32	(0.22)	0.00	(0.00)
Sebastes auriculatus	2	0.04	(0.04)	0.04	(0.04)	0.00	(0.00)
Scorpaenichthys marmoratus	7	0.00	(0.00)	0.21	(0.08)	0.04	(0.04)
Hexagrammos decagrammus	10	0.07	(0.05)	0.29	(0.16)	0.00	(0.00)
Pleuronichthys coenosus	6	0.14	(0.08)	0.04	(0.04)	0.04	(0.04)
Gibbonsia spp.	8	0.04	(0.04)	0.25	(0.08)	0.00	(0.00)
Sebastes dalli	5	0.14	(0.14)	0.04	(0.04)	0.00	(0.00)
Paralabrax clathratus	6	0.14	(0.14)	0.07	(0.05)	0.00	(0.00)
Coryphopterus nicholsii	6	0.00	(0.00)	0.21	(0.12)	0.00	(0.00)
Unidentified Cottidae	3	0.00	(0.00)	0.11	(0.08)	0.00	(0.00)
Leiocottus hirundo	2	0.04	(0.04)	0.04	(0.04)	0.00	(0.00)
Neoclinus blanchardi	4	0.07	(0.05)	0.07	(0.05)	0.00	(0.00)
Paralichthys californicus	4	0.00	(0.00)	0.07	(0.05)	0.07	(0.07)
Sebastes atrovirens	1	0.04	(0.04)	0.00	(0.00)	0.00	(0.00)
Syngnathus spp.	3	0.04	(0.04)	0.04	(0.04)	0.04	(0.04)
Sebastes carnatus	1	0.00	(0.00)	0.04	(0.04)	0.00	(0.00)
Cephaloscyllium ventriosum	1	0.00	(0.00)	0.04	(0.04)	0.00	(0.00)
Halichoeres semicinctus	1	0.04	(0.04)	0.00	(0.00)	0.00	(0.00)
Heterodontus francisci	1	0.00	(0.00)	0.04	(0.04)	0.00	(0.00)
Myliobatis californica	1	0.00	(0.00)	0.00	(0.00)	0.04	(0.04)
Phanerodon atripes	1	0.00	(0.00)	0.00	(0.00)	0.04	(0.04)
Unidentified Embiotocidae	1	0.00	(0.00)	0.00	(0.00)	0.04	(0.04)
All fishes	1758	26.86	(4.05)	26.68	(3.47)	9.89	(2.15)

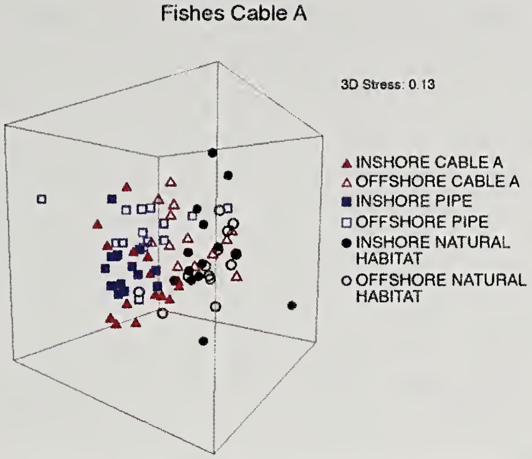

Fig. 5. A 3-d multiple dimensional scaling model comparing the fish assemblages from shallower and deeper transects in Cable A, pipe, and natural habitat.

istius frenatus, *Phanerodon furcatus*, and *Cymatogaster aggregata*), young-of-the-year (YOY) *Sebastes* that had newly settled out of the plankton (particularly *Sebastes chrysomelas*, *S. carnatus*, and *S. atrovirens*), and relatively solitary substrate-oriented species (i.e., *Citharichthys* spp.). *Oxyjulis californica*, *Citharichthys* spp., *Phanerodon furcatus*, YOY *Sebastes*, and *B. frenatus* were the most abundant taxa. *Cables*: At least 35 species and 1,661 individuals were observed over the energized cables. *Oxyjulis californica*, *Citharichthys* spp., *B. frenata*, *P. furcatus*, and YOY *Sebastes* were most abundant (Tables 4, 5). *Pipe*: The number of taxa (37) and individuals (1,712) were similar to those observed on the cables. *Oxyjulis californica*, YOY *Sebastes*, *Sebastes miniatus* YOY, *Damalichthys vacca*, *Embiotoca jacksoni*, and *Citharichthys* spp. were the most abundant taxa on the pipe (Tables 4, 5). *Natural Habitat*: Fewest species (25) and individuals (1,092) were observed over the natural habitat. *Cymatogaster aggregata*, *Citharichthys* spp., *O. californica*, YOY *Sebastes jordani*, *P. furcatus*, and *Aulorhynchus flavidus* were the most commonly observed species (Tables 4, 5).

Fish communities among all habitats were composed primarily of small-sized fishes with most being less than 20 cm long. The mean lengths of fishes (cables = 11.8 cm, pipe = 11.4 cm, natural habitat = 9.7 cm) varied significantly among the three habitats (Welch's Test, $F = 43.7$, $df = 2$, $p < 0.0001$) as did the size distributions (Kolmogorov Smirnov Two-Sample Test: cables versus pipe, $N = 3,484$ KS 0.053, $p = <0.0001$; cables versus natural habitat, $N = 2,832$ KS 0.147, $p = <0.0001$; pipe versus natural habitat, $N = 2,890$ KS 0.117, $p = <0.0001$).

While the overall fish communities were similar among the three habitats, there were some fish species that were statistically more abundant over parts of either the cables or pipe (Table 3, Fig. 6). As examples, *O. californica*, *Citharichthys* spp., and *E. jacksoni* were all more abundant over Cable B than over the pipe (Table 3, Fig. 6). Similarly, *B. frenatus*, *Sebastes paucispinis* YOY, and *Heterostichus rostratus* were more abundant over Cable A compared to the pipe. However, with the exception of *Citharichthys* spp., none of these species were *consistently* more abundant at either the cables or the pipe or over both depths. Rather, in virtually all of these instances these differences were either 1) limited to one of the two cables or 2) were not consistent between depths (Fig. 6). As an example, while *O. californica* was statistically more abundant at Cable B than at the pipe, it was less abundant at Cable A compared to the pipe. Similarly, *Hypsurus*

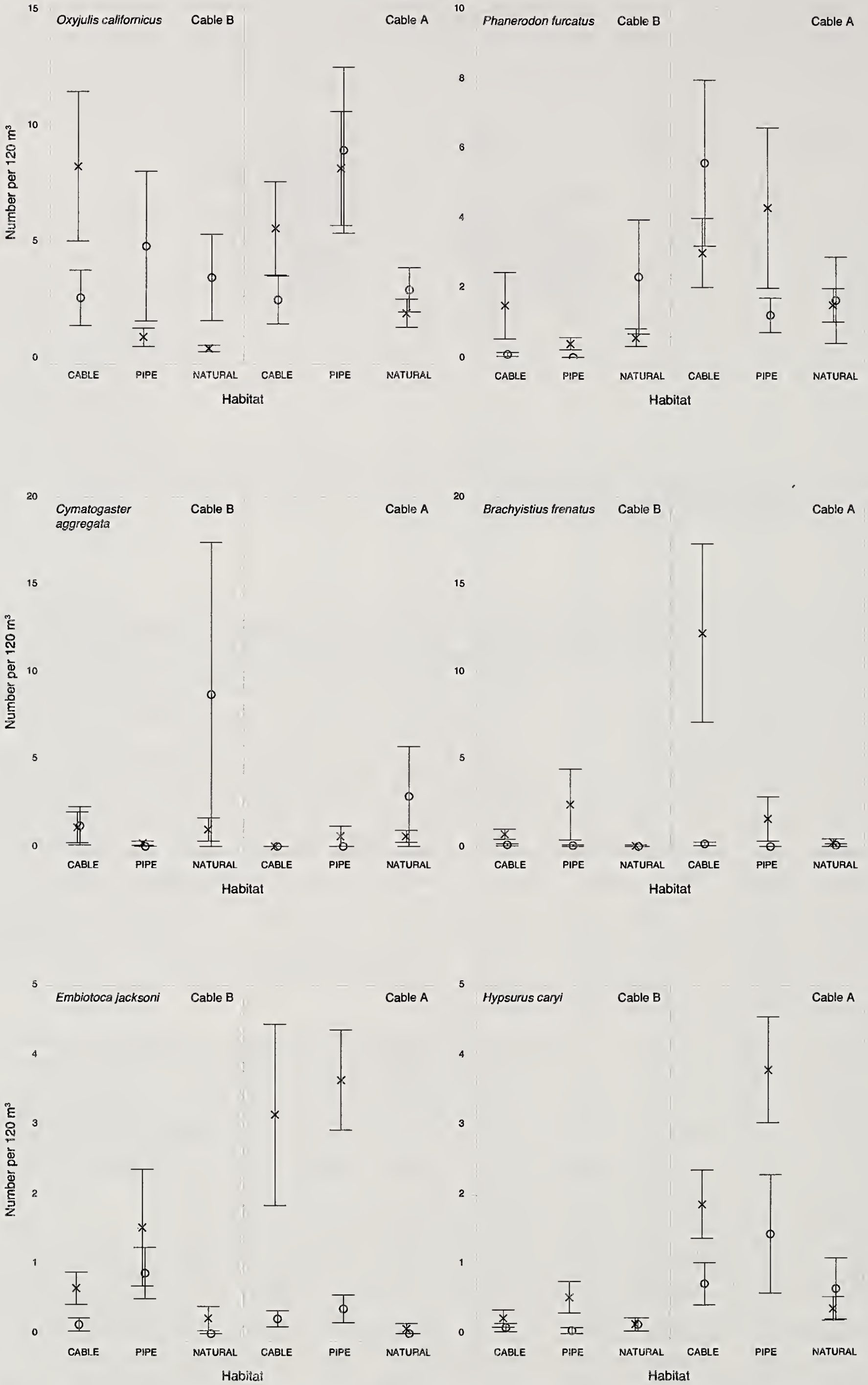

Fig. 6. Mean fish species densities found at the shallower and deeper sites at the three habitats. Data is divided into two periods when 1) Cable B was surveyed and 2) when Cable A was surveyed. KGB = young-of-the-year *Sebastes atrovirens*, *S. carnatus*, *S. chrysomelas*, and *S. caurinus*.

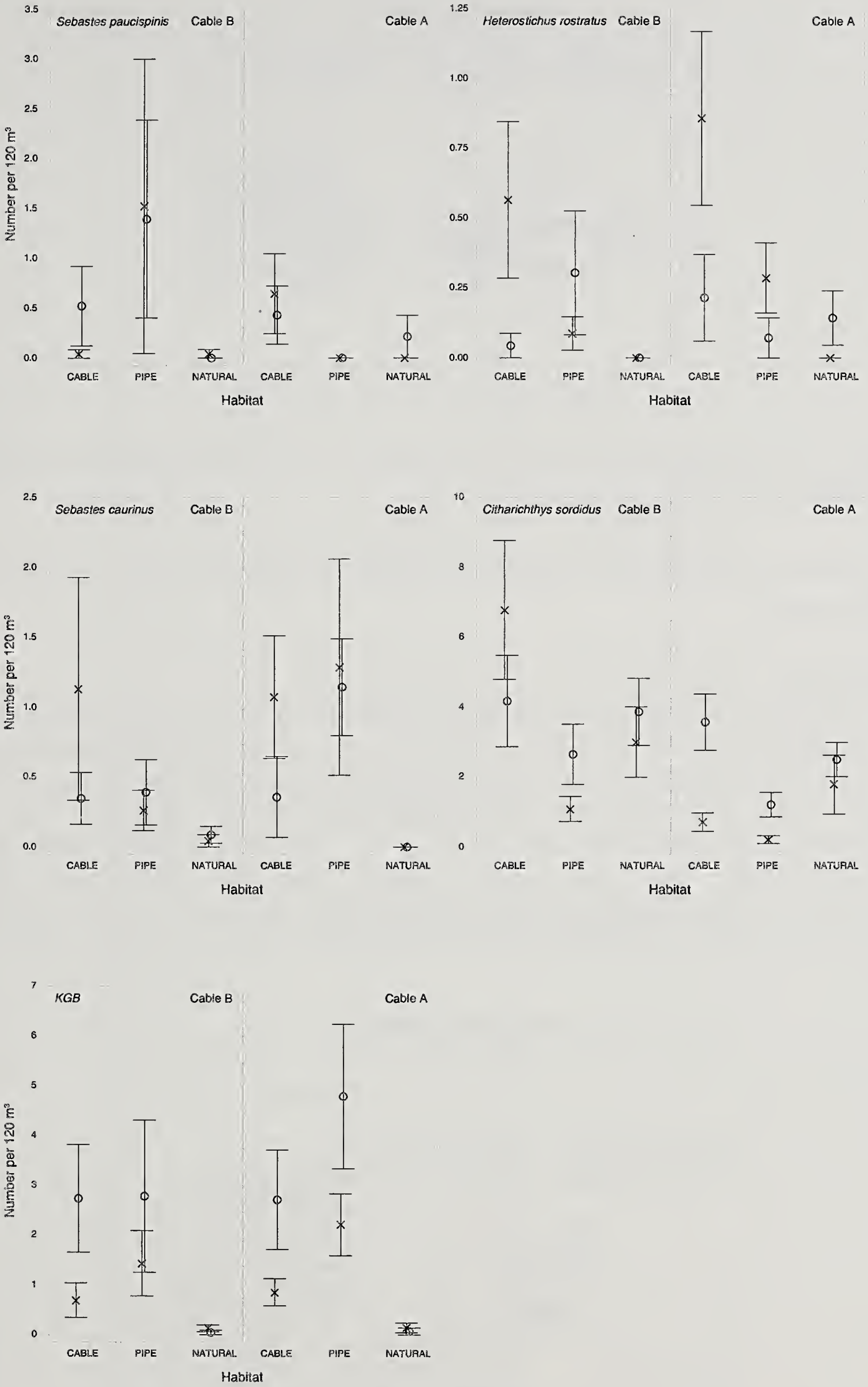

Fig. 6. Continued.

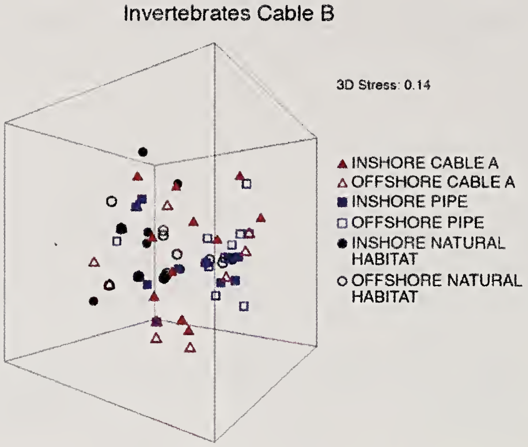

Fig. 7. A 3-d multiple dimensional scaling model comparing the invertebrate assemblages from shallower and deeper transects in Cable B, pipe, and natural habitat.

caryi were more abundant on the inshore parts of the pipe compared to Cable A, but not on the offshore parts.

Invertebrates

As with the fish community, the invertebrate assemblages varied among the cables, pipe, and natural habitat with significant interaction between the effects of habitat and depth on assemblage structure (Table 6). The three-dimensional MDS plots of the assemblages from shallow and deep transect samples demonstrates substantial overlap of the habitat groupings during the periods when both cable B (global R=0.085, p=0.002; Fig. 7) and cable A were surveyed (global R=0.227, p=0.0001; Fig. 8). We conducted a total of 30 days of invertebrate surveys during three years. A total of 802 individuals were observed, comprising at least 19 species (Tables 7, 8). *Patiria miniata*, several species of *Pisaster* sea stars, *Aplysia californica*, *Astropecten*

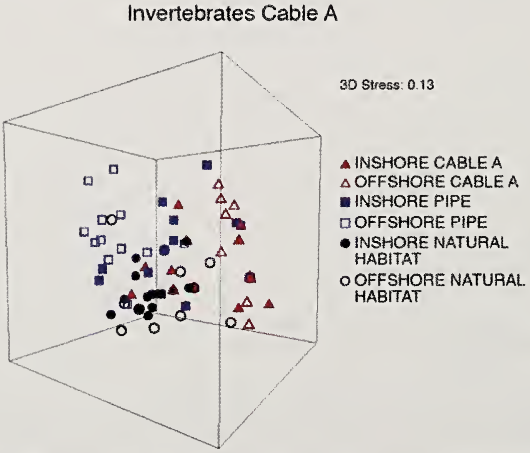

Fig. 8. A 3-d multiple dimensional scaling model comparing the invertebrate assemblages from shallower and deeper transects in Cable A, pipe, and natural habitat.

Table 6. P-values from the repeated measures PERMANOVA testing the effects of habitat (HA), depth (IN), and sampling date (DA) on invertebrate community structure and density of individual taxa and from PERMANOVA pairwise tests for differences between habitats for the periods when Cables B and A were surveyed. Species selected were those that comprised at least 1% of all invertebrates observed. Statistically significant effects and differences are underlined.

Cable B	HA	IN	DA	HAxIN	HAxDA	INxDA	Cable, pipe	Cable, natural	Pipe, natural
Invertebrate community	0.0017	0.2021	0.0238	0.0365	0.4545	0.1237	0.0354	0.0610	0.0061
Taxon:									
Pisaster spp.	0.0011	0.2884	0.0123	0.2881	0.0532	0.3558	0.0196	0.1248	0.0038
Patiria miniata	0.1528	0.9272	0.6332	0.0046	0.2595	0.1744			
Aplysia californica	0.0327	0.4780	0.1149	0.5209	0.2109	0.0716	0.6433	0.0455	0.0020
Kelletia kelletii	0.1097	0.1175	0.1485	0.6568	0.6353	0.0738			
Parastichopus spp.	0.3903	0.3395	0.3746	0.4344	0.5065	0.4964			
Metacarcinus spp./*Cancer* spp.	0.0303	0.4645	0.7884	0.0338	0.8292	0.3765	0.0650	0.0423	0.8462
Cable A	HA	IN	DA	HAxIN	HAxDA	INxDA	Cable, pipe	Cable, natural	Pipe, natural
Invertebrate community	0.0001	0.0054	0.0001	0.0044	0.2620	0.2436	0.0005	0.0001	0.0002
Taxon:									
Pisaster spp.	0.0001	0.0031	0.0015	0.0091	0.5799	0.1771	0.0009	0.1841	0.0003
Patiria miniata	0.0001	0.1323	0.0071	0.0008	0.1950	0.0064	0.0003	0.0002	0.3001
Astropecten armatus	0.0190	0.0205	0.0042	0.0573	0.1447	0.4878	0.3525	0.0098	0.0855
Aplysia californica	0.0007	0.2111	0.0141	0.7424	0.0036	0.8056	0.0174	0.0969	0.0009
Kelletia kelletii	0.2817	0.6565	0.2405	0.7408	0.7233	0.0881			
Parastichopus spp.	0.0001	0.0017	0.0246	0.0060	0.0233	0.5548	0.0004	not defined	0.0002
Metacarcinus spp./*Cancer* spp.	0.8382	0.3927	0.4557	0.8323	0.9260	0.9788			

Table 7. Total counts across all habitats and mean (SE) number of invertebrates in each habitat during period when Cable B was surveyed. Two transects, shallow and deep, were surveyed in each habitat on 22 June–15 May 2013 sampling dates. Number of transect surveys in each habitat, n=30.

Scientific name	Total count	CABLE B Mean	CABLE B SE	Pipe Mean	Pipe SE	Natural Mean	Natural SE
Patiria miniata	78	0.43	0.14	0.97	0.29	1.20	0.32
Pisaster spp.	70	0.73	0.31	1.27	0.25	0.33	0.14
Aplysia californica	19	0.33	0.12	0.27	0.11	0.03	0.03
Kelletia kelletii	27	0.37	0.19	0.03	0.03	0.50	0.22
Parastichopus sp.	1	0.00	0.00	0.03	0.03	0.00	0.00
Metacarcinus sp. *and Cancer* sp.	18	0.47	0.18	0.07	0.05	0.07	0.07
Loligo opalescens eggs	20	0.00	0.00	0.67	0.67	0.00	0.00
Pugettia spp.	18	0.30	0.13	0.27	0.14	0.03	0.03
Loxorhynchus spp.	12	0.10	0.06	0.13	0.06	0.17	0.08
Dendraster excentricus	19	0.10	0.07	0.03	0.03	0.50	0.28
Octopus spp.	2	0.03	0.03	0.00	0.00	0.03	0.03
Metacarcinus gracilis	5	0.07	0.07	0.07	0.07	0.03	0.03
Dermasterias imbricata	3	0.10	0.10	0.00	0.00	0.00	0.00
Megathura crenulata	1	0.00	0.00	0.03	0.03	0.00	0.00
Pycnopodia helianthoides	1	0.03	0.03	0.00	0.00	0.00	0.00

Table 8. Total counts across all habitats and mean (SE) number of invertebrates in each habitat during period when Cable A was surveyed. Two transects, shallow and deep, were surveyed in each habitat on 14 June 2013–26 February 2014 sampling dates. Number of transect surveys in each habitat, n=28.

Scientific name	Total count	CABLE A Mean	CABLE A SE	Pipe Mean	Pipe SE	Natural Mean	Natural SE
Patiria miniata	155	0.75	0.26	2.14	0.38	2.64	0.41
Pisaster spp.	92	0.46	0.17	2.61	0.66	0.21	0.08
Strongylocentrotus purpuratus	100	0.00	0.00	3.57	3.57	0.00	0.00
Aplysia californica	44	0.29	0.12	1.25	0.44	0.04	0.04
Astropecten armatus	51	0.29	0.10	0.43	0.12	1.11	0.37
Kelletia kelletii	21	0.04	0.04	0.46	0.33	0.25	0.15
Parastichopus sp.	21	0.04	0.04	0.68	0.18	0.04	0.04
Metacarcinus sp. *and Cancer* sp.	4	0.07	0.05	0.04	0.04	0.04	0.04
Pugettia spp.	1	0.00	0.00	0.00	0.00	0.04	0.04
Loxorhynchus spp.	7	0.04	0.04	0.11	0.06	0.11	0.08
Octopus spp.	10	0.07	0.07	0.29	0.10	0.00	0.00
Dermasterias imbricata	1	0.00	0.00	0.00	0.00	0.04	0.04
Panulirus interruptus	1	0.04	0.04	0.00	0.00	0.00	0.00

armatus, and *Kelletia kelletii* were observed most often. By group, sea stars were the most abundant, comprising 56.8% of all invertebrates observed. *Cables*: We recorded 157 individuals of at least 15 species at the cable sites. *P. miniata*, *Pisaster* sea stars, and *A. californica* were most abundant (Tables 7, 8). *Pipe*: A total of 422 individual invertebrates, more than any other site, were observed at the pipe. However, 100 of these individuals were comprised of a one-time recorded aggregation of *Strongylocentrotus purpuratus*. Like the cables, we recorded 15 species along the pipe (Tables 7, 8). *Natural Habitat*: *Patiria miniata* and *K. kelletii* predominated in the natural habitat, where we recorded 223 individuals, of 13 species (Tables 7, 8). Again, consistent with what we observed for fish species, in comparing cables with the pipe, only one invertebrate species was consistently more abundant in either habitat: *Pisaster* spp. were more abundant over the pipe (Table 8, Fig. 9). Several other species, such as *P. miniata*, *Parastichopus* spp., *A. californica*, and *Parastichopus* spp. were not consistently more abundant on either cables or pipe.

Macrophytes

In contrast to the fish and invertebrate communities, the macrophyte assemblages were strikingly distinct from one another by habitat and depth. The interaction between these two effects on assemblage structure was significant (Table 9). The 3-dimensional MDS plots of the assemblages from shallow and deep transect samples demonstrates no overlap of the habitat groupings during the periods when Cable B (global R=0.998 p=0.0001; Fig. 10) and Cable A were surveyed (global R=0.993, p=0.0001; Fig. 11).

We conducted a total of 38 days of surveys during three years. A total of 76358 individual macrophytes (many likely observed repeatedly on sequential survey days) were tallied, comprising at least five species (Table 10). Overall, *Zostera marina* was most abundant (and found only on the natural habitat), followed by *Pterygophora californica*, *Cystoseira* spp., *Macrocystis pyrifera*, and *Laminaria* spp. *Cables*: Overall, *Pterygophora californica* dominated the cable community, although *Cystoseira* spp. and *Laminaria* spp. were not uncommon (Table 10). However, note that *P. californica* was very abundant on Cable B (particularly shallower), but much less so on Cable A (Table 10). *Zostera marina* grew on the sand near the cable. *Macrocystis pyrifera* grew very sparsely on the shallower Cable B habitat, was more common on the shallower part of Cable A, and was essentially absent from the deeper cables (Fig. 12). *Pipe*: *Cystoseira* spp. was the most common macrophyte on the pipe (Table 10). *Cystoseira* spp. was about twice as abundant shallower than deeper while *Laminaria* spp. was almost absent from the shallower site and nearly as abundant as *Cystoseira* spp. deeper (Fig. 12). Relatively few *P. californica* were observed on the pipe and both *M. pyrifera* and *Z. marina* were almost absent. *Natural Habitat*: *Zostera marina* was the only macrophyte growing on the sandy sea floor of the natural habitat (Table 10). It was dense at both the shallower and deeper sites (Fig. 12). As noted above, the macrophyte communities in the three habitats differed among each other and along the inshore and offshore transects. This was reflected in the distribution of all macrophyte species when comparing cable and pipe habitats (Table 10). Unlike with virtually all of the fish species and all of the invertebrate species, the differences in abundances were consistent between both cables and the pipe and at both depths (Fig. 12).

Discussion and Conclusions

We began this study with the understanding that if a species is attracted to an EMF we would expect to find that species in disproportionately larger numbers or densities around the energized cables compared to the pipe or natural habitat. Similarly, if a taxa is repelled by that EMF we

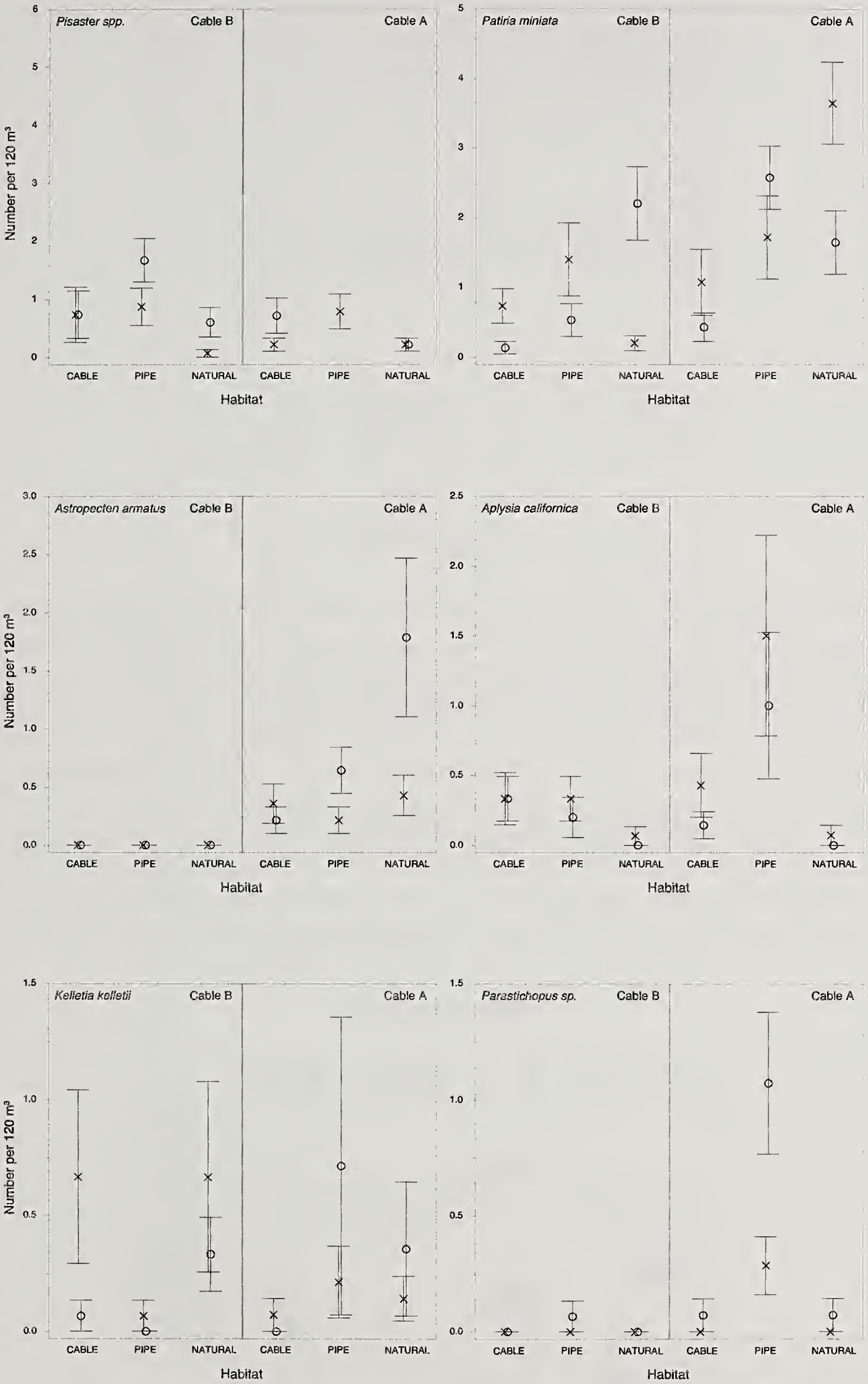

Fig. 9. Mean invertebrate species densities found at the shallower and deeper sites at the three habitats. Data is divided into two periods when 1) Cable B was surveyed and 2) when Cable A was surveyed.

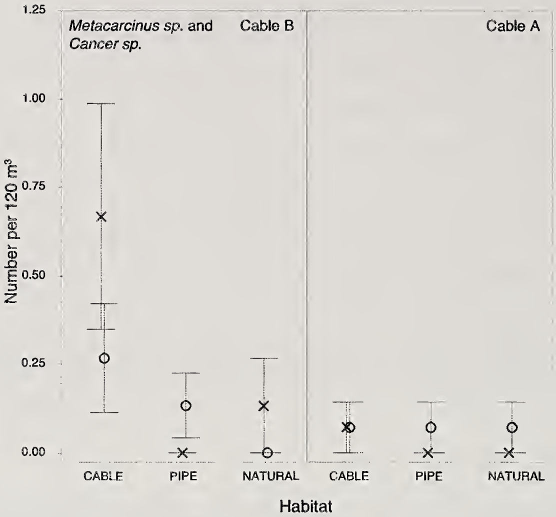

Fig. 9. (Continued).

would expect that species to be present less often or in lower densities at the cables. However, the presence or absence of an EMF is not the only habitat parameter influencing how an organism chooses its habitat. We acknowledge that in this study the cables and pipe differed not only in the production of an EMF but to some extent in the morphology of these habitats. In particular, the pipe was a slightly more complex structure. First, the pipe's diameter (30.48 cm) was somewhat greater than that of the two cables (20.32 cm), and while the cable was sometimes partially buried, the pipe was not. Thus for both reasons the pipe tended to present a somewhat higher profile. In addition, perhaps the greatest structural difference between the cables and pipe was the very high density, particularly on the shallower pipe, of *Cystoceira* sp., a brown alga that was essentially absent from the shallower cable. This alga forms a dense cover near the bottom and small fishes, particularly YOY *Sebastes*, will preferentially inhabit this complex substratum.

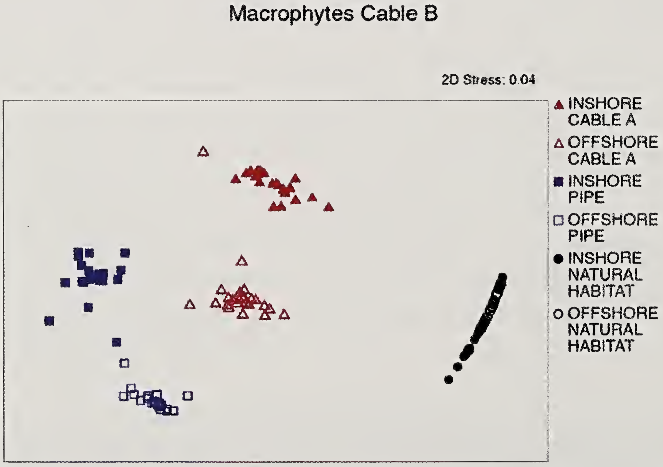

Fig. 10. A 2-d multiple dimensional scaling model comparing the macrophyte assemblages from shallower and deeper transects in Cable B, pipe, and natural habitat.

Table 9. P-values from the repeated measures PERMANOVA testing the effects of habitat (HA), depth (IN), and sampling date (DA) on macrophyte community structure and density of individual taxa and from PERMANOVA pairwise tests for differences between habitats for the periods when Cables B and A were surveyed. Statistically significant effects and differences are underlined.

Cable B	HA	IN	DA	HAxIN	HAxDA	INxDA	Cable, pipe	Cable, natural	Pipe, natural
Macrophyte community	0.0001	0.0001	0.0001	0.0001	0.0003	0.0002	0.0001	0.0001	0.0001
Taxon:									
Zostera marina	0.0001	0.2553	0.0659	0.3820	0.1259	0.3389	0.0001	0.0001	0.0001
Pterygophora californica	0.0001	0.0001	0.0676	0.0001	0.1672	0.5020	0.0001	0.0001	0.0001
Cystoseira spp.	0.0001	0.0026	0.0019	0.0001	0.0001	0.1156	0.0001	0.0001	0.0001
Laminaria spp.	0.0001	0.0001	0.1713	0.0001	0.0574	0.0395	0.0037	0.0001	0.0001
Cable A	HA	IN	DA	HAxIN	HAxDA	INxDA	Cable, pipe	Cable, natural	Pipe, natural
Macrophyte community	0.0001	0.0001	0.0477	0.0001	0.0054	0.2401	0.0001	0.0001	0.0001
Taxon:									
Zostera marina	0.0001	0.0001	0.0215	0.0001	0.0720	0.4107	0.0001	0.0001	0.0001
Pterygophora californica	0.0001	0.0001	0.6193	0.0001	0.5937	0.1637	0.0001	0.0001	0.0001
Cystoseira spp.	0.0001	0.2970	0.0762	0.0001	0.6016	0.2163	0.0001	0.0001	0.0001
Laminaria spp.	0.0001	0.0001	0.8647	0.0001	0.9155	0.2615	0.2039	0.0001	0.0001
Macrocystis pyrifera	0.0001	0.0001	0.0654	0.0001	0.5037	0.0599	0.0001	0.0001	0.0001

Table 10. Total count across all habitats and mean (SE) number of macrophytes per transect in each habitat during period when cable B and cable A were surveyed, 1 February 2012—15 May 2013 and 14 June 2013–26 February 2014, respectively. Two transects, shallow and deep, were surveyed in each habitat on each date. Number of transect surveys in each habitat when Cable B and Cable A were surveyed, n=46 and n=28, respectively.

Cable B	Total Count	Cable Mean	Cable SE	Pipe Mean	Pipe SE	Natural Mean	Natural SE
Zostera marina	19453	16.96	3.41	0.00	0.00	405.93	28.21
Pterygophora californica	16490	344.98	36.11	13.50	2.55	0.00	0.00
Cystoseira spp.	5223	15.65	2.50	97.89	6.02	0.00	0.00
Macrocystis pyrifera	329	3.54	1.37	3.61	1.37	0.00	0.00
Laminaria spp.	2123	18.26	3.05	27.89	4.34	0.00	0.00
Cable A	Total Count	Cable Mean	Cable SE	Pipe Mean	Pipe SE	Natural Mean	Natural SE
Zostera marina	22791	14.36	2.97	0.07	0.07	799.54	50.88
Pterygophora californica	886	20.29	1.11	11.36	2.33	0.00	0.00
Cystoseira spp.	2943	17.11	1.97	88.00	8.43	0.00	0.00
Macrocystis pyrifera	5228	163.07	33.66	23.64	5.14	0.00	0.00
Laminaria spp.	892	12.39	2.61	19.46	3.80	0.00	0.00

Algae also grew on the cable, particularly *Macrocystis pyrifera* on the shallower area of Cable A, and *Laminaria* sp. on the deeper portion of both cables. However, *M. pyrifera* does not form luxuriant bottom structures and the *Laminaria* stands, while present, did not present as dense a cover as the *Cystoceira* on the pipe. The sandy natural habitat was the least complex of all three; its two-dimensional aspect was only broken up by stands of *Z. marina*. At the start of the study *Z. marina* was only sporadically found and became more abundant over time.

Structural variability aside, the results of our study demonstrated that the fish and invertebrate assemblages of the three habitats were similar. Although a few species statistically varied in abundance between the cables and pipe, in no instance was a fish or invertebrate species extremely abundant at one of these two habitats and extremely rare or absent from the other. And although fishes were statistically larger at the pipe than at the cable or natural habitat, we argue that this difference (of less than one-half centimeter between pipe and cable and two centimeters between pipe and natural habitat) is not biologically meaningful.

Results of this study found no evidence that any species of fish or invertebrate was either preferentially attracted to, or repelled by, the EMF emitted by the cables. Any observed differences in the fish or invertebrate densities between cables, pipe, and natural habitat taxa are most likely due to the differences in the physical characteristics of these habitats. For example, the higher densities of YOY *Sebastes* and *E. jacksoni* at the pipe are most likely due to greater densities of understory algae, specifically *Cystoseira* spp. In addition, the lower-relief cables, which were closer to the sandy sea floor, were a better habitat for soft-bottom dwelling sanddabs. Contrary to the fish and invertebrate assemblages, the plant communities on cables, pipe, and natural habitat were clearly different from one another. However, if cable EMF were responsible for these differences, we would expect to see similarities in plant communities between energized cables A and B and this was not the case. Rather, it appears that plant communities were driven by site depth (particularly among the algae) and habitat type (i.e., eelgrass).

We note that this study was not designed to directly determine the behavior of fishes and invertebrates when these organisms encounter an energized cable during, for instance, migrations. Rather, we observed the integration over time of myriads of such behaviors by many organisms. Understanding how individuals within a taxon relate to energized cables would have to involve

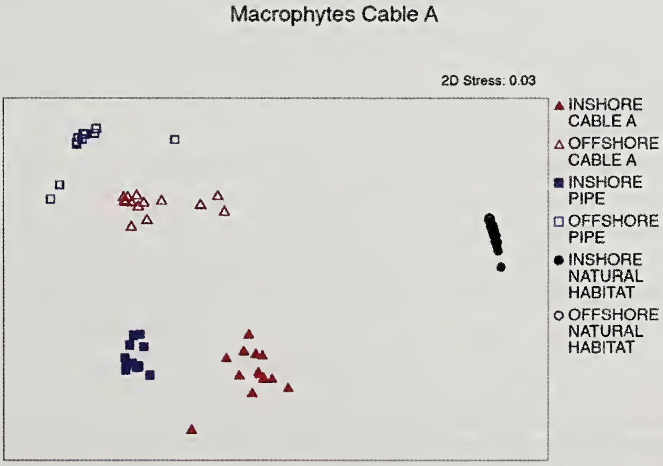

Fig. 11. A 2-d multiple dimensional scaling model comparing the macrophyte assemblages from shallower and deeper transects in Cable A, pipe, and natural habitat.

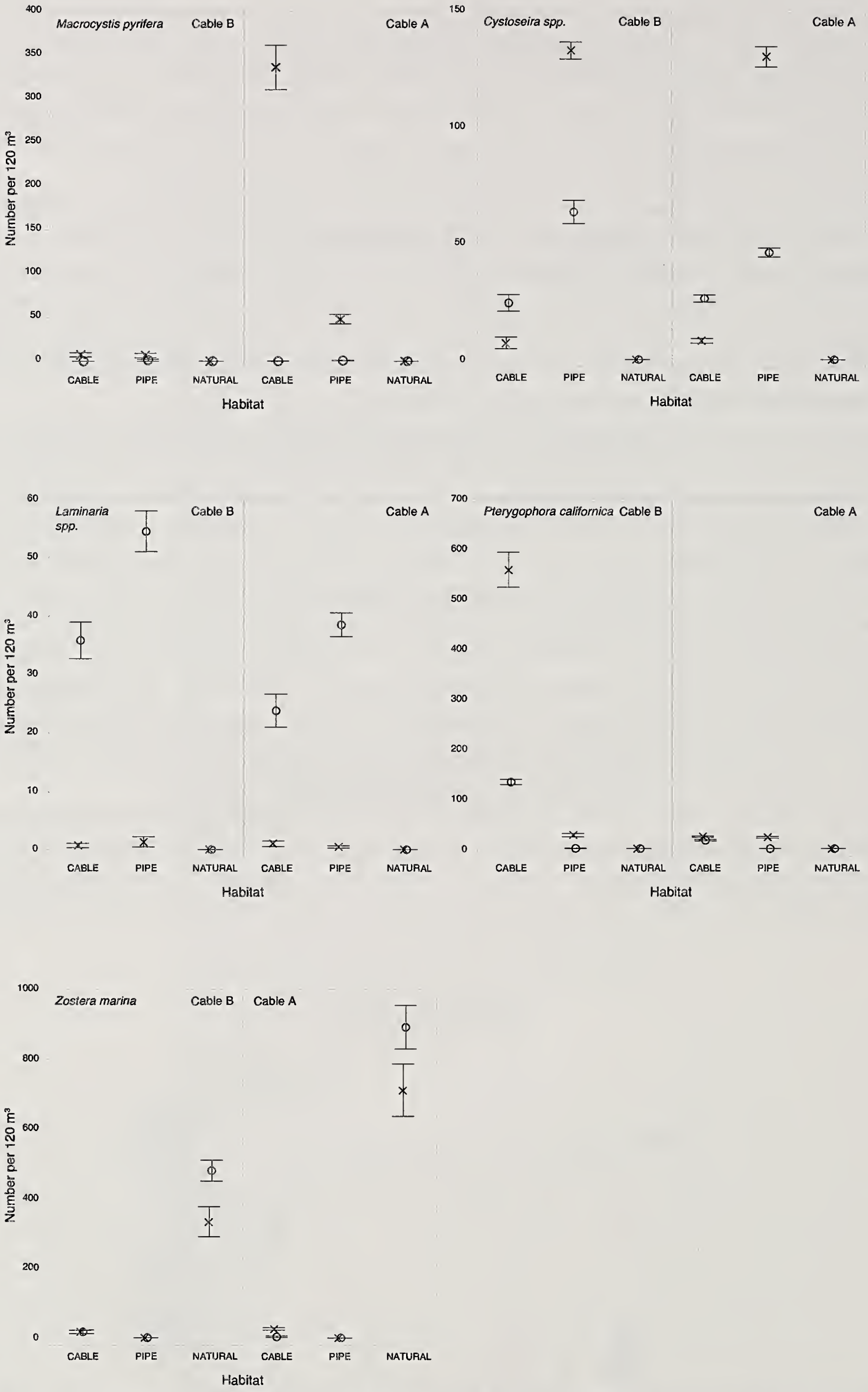

Fig. 12. Mean macrophyte species densities found at the shallower and deeper sites at the three habitats. Data is divided into two periods when 1) Cable B was surveyed and 2) when Cable A was surveyed.

either tracking (Westerberg and Lagenfelt 2008) or caging experiments (Love et al. 2015) or hybrids of the two (Gill et al. 2009).

In southern California, most along-shore migrations (as distinct from less synchronized movements) are conducted by such pelagic species as *Prionace glauca* and *Sardinops sagax*. The more substrate-associated shallower species (exemplified by the taxa that dominated our survey) tend to be either resident (i.e., *Cephaloscyllium ventriosum*, *E. jacksoni*), make seasonal shallower-deeper movements (*H. caryi*), or locally disperse as they mature (YOY *Sebastes* spp., *C. aggregata*). Given that the EMF emitted from the study cables is undetectable beginning at a distance of about one meter (Love et al. 2015, Love unpubl. data) it would be unlikely that pelagic and midwater species are affected by this field. In fact, the limited range of the EMF implies that only the movements of those species that live close to the bottom would be potentially impacted.

In our study area, some of the bottom-dwelling or bottom-oriented species most likely to respond to energized cables are the elasmobranchs: the sharks, skates, and rays. It is probable that all of these fishes can detect an EMF and this ability appears to be used for a number of behaviors including migration and food detection (Kalmijn 1971, Tricas 1982, Klimley et al. 2005). Moreover, while the actual sensitivity to an EMF is known for only a few elasmobranch species, we note that at least two Atlantic species, *Carcharhinus plumbeus* and *Sphyrna lewini*, are able to detect an EMF in the 25–100μT range (Meyer et al. 2005); this is within the range generated by the current surveys' energized cables.

The shallower habitats of southern California, and specifically this study site, harbor a rich diversity of elasmobranchs (Love 2011). These include both mobile taxa (e.g., *Triakis semifasciata* and *Mustelus* spp.) and more sedentary species (*Rhinobatos productus*, *Platyrhinoidis triseriata*, and *Squatina californica*). Given this diversity, it is interesting to note that over the course of this study we observed only two elasmobranch individuals, *C. ventriosum* near the pipe and *Urobatis halleri* near Cable B. It might be argued that the chances of seeing individuals of the more motile species would be small on any given day; although these chances would likely be increased if the animals were attracted to the cables. However, if the more sedentary species were similarly attracted, one might expect to have encountered them. And again, the absence of these animals from the cable is likely not because the EMF generated is below their sensory threshold. Rather, the data strongly imply that of the electro-sensitive species in the study area, at least the elasmobranchs are not attracted to the energized cables.

Our findings are particularly important because, worldwide, the small number of field or semi-field studies that have been conducted on how fishes respond to energized power cables have found either little or no response (Westerberg and Lagenfelt 2008, DONG Energy and Vattenfall A/S 2006, Love et al. 2015, present study) or, arguably, an equivocal one (Gill et al. 2009). One possible explanation is that marine organisms respond to human-made EMF differently from those produced in nature. Recent studies demonstrate that human-made EMF is inherently different from naturally produced EMF, with naturally produced EMF being polarized and consequently more biologically active (Panagopoulos et al. 2015). Thus, it is possible that electro-sensitive organisms are able to differentiate between the two types and therefore respond differently to each of these stimuli.

Regarding the specific objectives of this study:

1) *Differences exist among fish and invertebrate communities associated with energized and unenergized cable habitat and those communities in soft seafloor habitats lacking cables.*

We did not find any biologically significant differences among fish and invertebrate communities between energized cables, pipe, and natural habitat. In particular, only three species of fish showed statistically significant, but slight, differences in densities between the cables and pipe. Plant communities did differ among habitats and within habitats between depths. These differences were almost certainly structure and depth, rather than EMF, related.

2) *Electro-sensitive species that are regionally important, such as sharks and rays, respond (via either attraction or repulsion) to the EMFs of an* in situ *power transmission cable.*

We observed two elasmobranch individuals, *C. ventriosum* near the pipe and *Urobatis halleri* near one of the two energized cables, during the course of this study. Thus, it would appear that the EMFs generated by energized cables are either unimportant to these organisms or that at least other environmental factors take precedence.

3) *The potential effectiveness of the commonly proposed mitigation of cable burial.*

Given the rapidity with which the EMF produced by the energized cables diminishes and the lack of response to that EMF by the shallower fish and invertebrates, cable burial would not appear necessary strictly for biological reasons. In this and similar cases, cable burial, at sufficient depth, would be an adequate tool to prevent EMF emissions from being present at the seafloor.

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116(2), 2017, pp. 88–97

Does Estuary Restoration Design Alter the Fine Scale Movements of Gray Smoothhounds (*Mustelus californicus*) in Southern California?

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Abstract.—Restored estuaries in southern California are limited in size and shape by fragmentation from human development, which can in turn restrict habitat use. Thus, it is important to assess how habitat design affects how fish use restored estuaries. Acoustic telemetry tracking from prior studies revealed that Gray Smoothhounds (*Mustelus californicus)* used primarily the eelgrass ecotone and warm interior waters in Bolsa Chica Full Tidal Basin (BCFTB), a 1.48 km^2 open-format marine dominated estuary. In this study, *M. californicus* utilized the Channel in Huntington Beach Wetlands Complex (HBWC), a smaller creek estuary. The Channel had more eelgrass than other available habitats but was also the coolest microhabitat, with temperatures below what *M. californicus* was found to select in BCFTB. Individuals may behaviorally thermoregulate by moving upstream, away from the HBWC Channel, during periods of incoming, cooler ocean water. *Mustelus californicus* translocated to different microhabitats within the HBWC selected the Channel habitat after the translocation regardless of where animals were released. Despite the large difference in available subtidal habitat between HBWC and BCFTB, no differences in patch size utilization distributions of *M. californicus* were observed. While individuals seem to shift between microhabitats based on temperature and eelgrass availability, the area size used by *M. californicus* appears to be the same within both sites despite the differences in overall size between sites. These results suggest that differences in microhabitat use may influence distribution patterns of *M. californicus* within each site, and therefore, shark abundance may vary with the restoration design (e.g. basin versus channel) and the size of the estuarine habitat. This information on habitat selection will be critical to planning future restorations on the Southern California coast.

Introduction

Restoration of lost or degraded estuarine habitat has become a strategy for recouping habitat loss and providing additional nursery habitat for fishes (Zedler and Langis 1991 Zedler 1996). Designs for restoration sites differ depending on the project's goals and are often limited by the space available for restoration. In turn, a number of environmental parameters that are influenced by restoration designs, including available subtidal habitat, tidal flow, vegetation cover, microhabitat (used here as a small scale environmental feature, such as an eelgrass bed or mudflat, within the larger estuary habitat) diversity, and average depth, vary greatly among sites, and can impact ecological function (Zedler 1996; Nicolas et al. 2010). Habitat size and availability has shown to shape the habitat use of fish in natural systems (Topping et al. 2005; Topping et al. 2006); thus, differences in microhabitat availability due to restoration design could also impact abundances of targeted commercial species (Fodrie and Mendoza 2006; Freedman et al. 2016).

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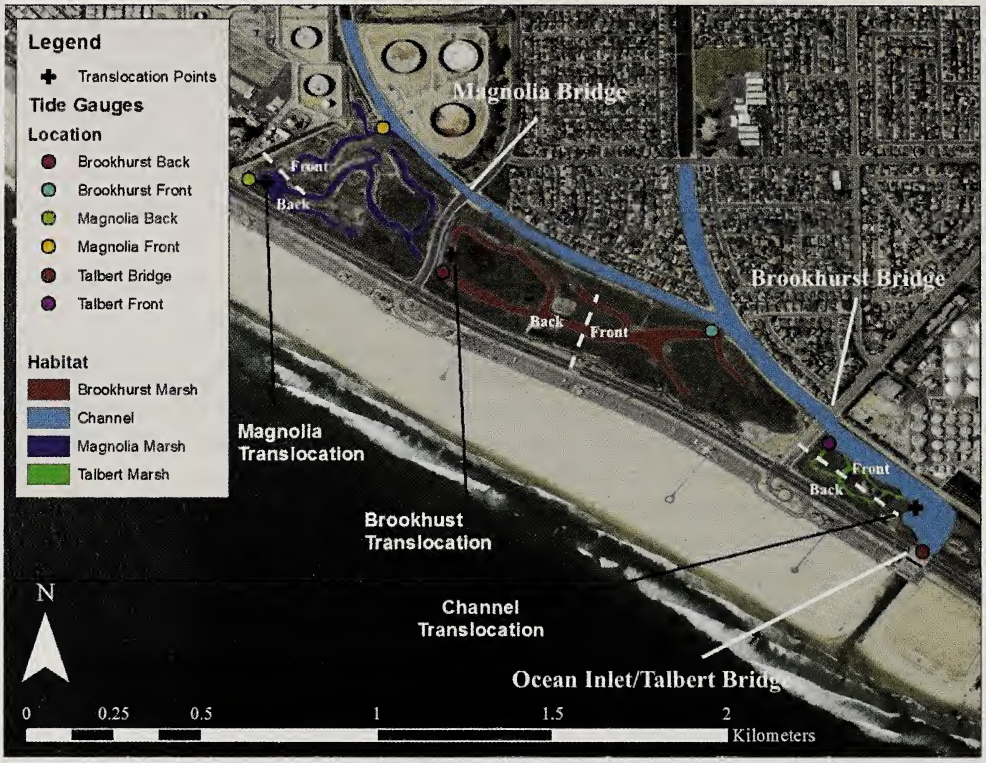
Two restored estuary habitat designs commonly used in southern California are tidal creek estuaries and full tidal basins. Tidal creek estuaries have narrow channelized aquatic microhabitats with relatively large intertidal mudflats interwoven with vegetated marsh plains. Comparatively, full tidal basins have larger continuous tracts of sub-tidal marine microhabitats, typically unbroken by intertidal habitats. Differences in microhabitats like intertidal mudflats, eelgrass beds, and deep channels likely affect habitat use within larger habitat complexes (e.g. tidal creek estuaries and full tidal basins). Generally, full tidal basins are thought to maximize available fish habitat, but it is unknown how various restoration designs impact habitat use of fishes. For example, vegetation on intertidal mudflats has been demonstrated to increase fish growth rates (Irlandi and Crawford 1997), and many predatory fishes have been found to selectively feed along and in this type of microhabitat (Carlisle and Starr 2009; Espinoza et al. 2011), which may make creek estuaries better suited for some fishes. The size, shape, and diversity of available habitat spaces have been shown to affect habitat utilization and movements of marine and estuarine fish species (Topping et al. 2005; Topping et al. 2006), and may therefore alter movements of coastal elasmobranchs while they are utilizing estuaries (Freedman et al. 2016; Huepel and Simpfendorder 2011; Carlisle and Starr 2009).

Many nearshore elasmobranch species from southern California are seasonal migrants, using primarily warmer and highly productive estuarine habitats relative to other cooler coastal habitats in the summer (Barry and Cailliet 1981; Knip et al. 2010; Espinoza et al. 2011; Farrugia et al. 2011; Jirik and Lowe 2012; Nosal et al. 2014). These summer conditions in the estuary can increase growth potential and survivorship, which leads juvenile elasmobranchs to seasonally select protective estuarine and bay habitat over exposed coastlines (Huepel and Hueter 2002; Espinoza et al. 2011; Farrugia et al. 2011; Huepel and Simpfendorder 2011). Despite their important role as nursery habitats for a variety of elasmobranchs (Huepel et al. 2007; Espinoza et al. 2011; Farrugia et al. 2011; Freedman et al. 2015), coastal wetlands in California have experienced a 90% decrease since 1850, mostly due to urbanization of coastlines (Zedler and Langis 1991; Zedler 1996; Larson 2001).

Compounding on the lack of available estuary habitat in the region, quality is not consistent across all estuaries (Fodrie and Mendoza 2006; Freedman et al. 2016). Although capture methods are not comparable quantitatively due to effort and gear differences, qualitatively, Catch Per Unit Effort (CPUE) of Gray Smoothhound, *Mustelus californicus,* were much higher in a full tidal basin (approximately 0.013 sharks per m^2; Espinoza et al. 2011) than in a tidal creek estuary (approximately 0.001 sharks per m^2; C. Whitcraft unpub. data). Differences in shark abundance between sites could be driven by microhabitat diversity, prey availability, and/or available habitat space and size between the sites. Restoration designs have the potential to alter available microhabitat types and habitat coverage as in natural systems, which in turn may alter fishes' behavior and habitat selection in differently designed restored estuaries. Because maximizing habitat use of fishes in restored estuaries is a common goal of restoration, understanding how designs alter habitat use is critical for coastal managers (Zedler 1996).


Legend
Translocation Points
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Brookhurst Back
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Magnolia Back
Magnolia Front
Talbert Bridge
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Talbert Marsh
Magnolia Bridge
Front
Back
Brookhurst Bridge
Magnolia Translocation
Brookhust Translocation
Channel Translocation
Ocean Inlet/Talbert Bridge
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0 0.25 0.5 1 1.5 2 Kilometers

Fig. 1. Study sites, with animal release locations/translocations in yellow and color-designated habitat divisions: (A) Bolsa Chica Full Tidal Basin, (B) study sites in relation to each other, (C) HBWC, and (D) the location of study sites along California.

To understand how habitat design could alter movements of a common coastal elasmobranch such as *M. californicus*, we collected movement data in a tidal creek estuary and compared it with sharks tracked by Espinoza et al. (2011) within a full tidal basin using a similar method. In addition, we performed a small-scale translocation experiment within the Huntington Beach Wetlands Complex (HBWC) to test the microhabitat site fidelity of *M. californicus*.

Materials and Methods

The Bolsa Chica Full Tidal Basin (BCFTB) and the Huntington Beach Wetlands Complex in Huntington Beach, CA are two restored estuaries in southern California situated approximately 10 km apart (Fig. 1). BCFTB is a 1.48 km^2 full tidal basin with a 4 m maximum depth that was opened to coastal waters in 2006. HBWC is a 0.77 km^2 tidal creek estuary composed of three distinct tidal creek marshes: a fully-draining creek system created in 1989 (Talbert Marsh), a 1.8 m deep fully-inundated creek opened to tidal flushing in 2009 (Brookhurst Marsh), and a small tidal basin with connecting marsh creeks that opened to tidal flushing in 2011 (Magnolia Marsh). All marshes are connected to each other and to the ocean via an armored flood control channel (hereafter "the Channel"). The HBWC marsh system and the BCFTB are composed primarily of mud and fine sediments, while the HBWC Channel is dominated by sand and shell hash. Eelgrass (*Zostera marina*) habitat, which can increase prey biomass (Kimmer et al. 1998; Leonard et al. 1998), was present but not evenly distributed in both the BCFTB and HBWC. In the HBWC, eelgrass was most prominent in the Channel microhabitat, with only a few small patches of eelgrass in the marsh creeks at the time of the surveys and translocations. In BCFTB, eelgrass was mostly found in the deep center of the basin, closer to the ocean inlet, throughout the duration of the study.

Two Ruskin tide gauges (RBR Limited, Model TGR-2050P, 0-10 m working depth) were deployed for one-month intervals at six rotating locations in HBWC (Fig. 1) from 2009-2013 to record water level and temperature every 10 min. The rotation among stations meant that while two stations had a gauge for a one-month interval, the others were empty until the gauges were moved. Probes were placed within 10 cm of the bottom. "Front" stations were located at the interface between the Marshes and Channel, while stations interior of the Marsh were designated as "back." Because Talbert fully drains at low tide, there was no Talbert "back" station. "Talbert Bridge" was placed under the Pacific Coast Highway bridge, approximately 300 m inland of the ocean inlet. Since *M. californicus* typically use estuaries during the summer months, daily mean summer temperature data (May to September) were calculated and compared among locations using Generalized Linear Mix Effect Model (GLMM), with "Date" as a random blocking factor.

To identify microhabitat use by *M. californicus*, the HBWC was divided into 6 major categories: Lower Channel (from Brookhurst Bridge to the ocean inlet), Middle Channel (From Brookhurst Bridge to Magnolia Bridge), Upper Channel (from Magnolia St. Bridge and beyond), Magnolia Marsh creek, Brookhurst Marsh creek, and Talbert Marsh creek (Fig. 1). These divisions were made based on expert judgment using estimated tidal flushing as assumed from distance from the mouth, and temperature (Freedman et al. 2016, Whitcraft unpub. data). BCFTB has no divisions, as the estuary was designed to maximize subtidal space once opened to tidal flushing in 2006. Ocean tidal height data were collected from the nearest NOAA tide station each minute (Los Angeles 9410660 NOS/CO-OPS) and used in analysis of movement data. Tidal height was used as a proxy for ocean temperature because temperature data was not available spatially throughout both estuaries, and tidal height would be available for both sites at their respective time periods. Data from monitoring programs in HBWC show a strong relationship between temperature and tidal height (Whitcraft unpub. data, Freedman et al. 2016).

Mustelus californicus were collected in both study sites using a 100 m long polyethylene longline with 3 m long monofilament line (36 kg test) and a barbless circle hook (Mustad #4/0-5/0) baited with market squid. Once *M. californicus* were captured, total length was measured and individuals were held in coolers of fresh seawater until tagging. Individuals over 55 cm FL were inverted to induce tonic immobility before surgical implantation of coded acoustic transmitters. Shark sizes were comparable between BCFTB (average size = 68.47 cm TL, range = 60.2 cm −101.4 cm TL) and HBWC (average size = 69.53 cm TL, range = 55.1 cm - 90 cm TL). Acoustic transmitters (VEMCO, V9-1L, 29 mm long, power output = 145–151 dB, battery life = 14 d, pulse interval = 2 s, frequency range = 63–84 kHz) were placed in the body cavity via a 1 cm incision along the ventral midline. The incision was closed with two sutures (Ethicon Chromic Gut 2–0) and then sharks were kept in seawater until they resumed normal swimming behavior. All animal handling and surgical procedures were approved by the CSULB IACUC (#254, 290).

In the BCFTB, coded acoustic transmitters (VEMCO V13-1L-R64k, 69 kHz, 40–80 s pulse interval, estimated battery life = 700 d) and 16 VR2W omni-directional underwater acoustic receivers in a VEMCO Position System array were used to assess the fine-scale movements of *M. californicus* (n = 22) in 2008 and 2009 (see Espinoza et al. 2011 for methods). The narrow width of the HBWC channels prevented an effective VPS system in that site, so we used an active acoustic tracking approach to collect similar fine-scale data.

Between June 2013 and October 2014, sharks were tracked in the HBWC following a translocation manipulation to test microhabitat associations determined by prior work in BCFTB. Sharks were captured in both the Channel (n = 4) and Magnolia Marsh Creek (n = 4) and translocated between Magnolia Marsh Creek and the Channel within HBWC (See Fig. 1 for translocation positions). Individuals were fitted with an acoustic transmitter (V9-1L, 29 mm long, power output = 145, battery life = 14 d, freq. pulse intervals = 2 s), translocated, and manually tracked continuously for 24 h from a vessel-based VR100 (VEMCO, Inc.) with a directional hydrophone immediately upon translocation. Three to four days after the initial 24 h track ended, *M. californicus* were located and tracked a second time for an additional continuous 24 h period. During these second tracks, sharks were assumed to have returned to their normal "pre-translocated" behavior.

Tracking geopositions for sharks from both studies were loaded in R (R Development Core Team 2013) and randomly sub-sampled over 24 h periods to make the data comparable between methods. A Biased Random Bridge analysis from the ADEHabitatHR package (Calenge 2006) was used to generate 50% and 95% habitat space utilization distributions for fish location in each 24 h period. The core area was defined as the 50% extent of a shark spatial distribution in a 24 h period. Daily activity was defined as the 95% extent of the area used by a shark in a given day. To test whether space use size was different between sites, habitat utilization areas were compared between *M. californicus* in BCFTB and HBWC using a Mann-Whitney U-test. Because individuals in HBWC experienced translocation, we only used movements of fish from the second track that were assumed to represent normal behavior movements and unaffected by a translocation manipulation.

First-time passage analysis (FTP) was used to compare estimated foraging patch size between sites. In FTP, radii with the highest variances for the log of the passage time are assumed to be the estimated spatial scale at which an animal searches for resources, or the patch size. The radii of patch, as an estimate of patch use size, were compared between *M. californicus* tracked in BCFTB and those of individuals in HBWC using a Mann-Whitney U test.

Temperature was previously found to play a major role in *M. californicus* habitat selection (Espinoza et al. 2011). However, in the HBWC, tidal flushing can more drastically alter the water temperature and temperature fluctuations in comparison with BCFTB due to shallower depth and the narrow channelization of HBWC. General Additive Models (GAMs, R package 'gam') were used to test the effect of tide on habitat selection by sharks within HBWC and BCFTB. The distance of a shark to the estuary mouth was determined by the Euclidian distance to the ocean inlet, whereas tide was measured as tidal height. We used distance from ocean inlet because it is assumed that the amount of tidal flushing, and therefore the magnitude of influx of cooler water, in a habitat is inversely related to its distance from shore.

Results

There was no difference in the amount of space used by sharks tracked in HBWC and BCFTB using both 95% and 50% utilization distributions from Biased Random Bridges (Table 1, W = 64, p = 0.84 for 95% utilization and W = 76, p = 0.35 for 50% utilization). In addition, patch use size from FTP was not significantly different between the HBWC and BCFTB (Mann-Whitney U, W = 36, df = 2, p = 0.098).

All *M. californicus* translocated to Magnolia Marsh Creeks from the Channel utilized the marsh for the full 24 h period after translocation, but were all found in the Channel two to three days following the translocation. During the second 24 h track in the Channel, *M. californicus* were typically found to remain in the Channel for the whole 24 h track. *Mustelus californicus*

Table 1. The 95% and 50% utilization distributions are in the table below. The sizes of both utilization distributions are similar in both sites, regardless of available subtidal habitat area.

Location	95% Utilization distribution in km^2 (median, range)	50% Utilization distribution in km^2 (median, range)
BCFTB	1.12, 0.22 – 11.12	0.19, 0.15 - 0.19
Channel	1.29, 0.12 - 7.71	0.12, 0.03 - 1.00

captured in Magnolia Marsh Creeks that were translocated to the Channel mostly used the Channel in the first 24 h after translocation, as well as two to three days after translocation. During the assumed return to normal behavior in the HBWC, *M. californicus* spent longer periods of time near the Lower Channel during both night and day time periods compared to any other available microhabitat in HBWC ($X^2 = 1569.35$, df = 4, $p < 0.001$ for day; $X^2 = 456.05$, df = 4, $p < 0.001$ for night). During high tides, *M. californicus* moved farther back into the HBWC Channel and marsh creeks. As tidal height went up, water temperatures would fall, especially in the Channel near the ocean inlet. Tidal height was significantly related to distance to estuary mouth in HBWC (Fig. 2, GAM, df = 3, Npar = 5.27, Pr(F) = 0.0013, p = 0.001). A similar relationship between tide and distance from mouth was also found in BCFTB (GAM, df = 3, Npar = 19.42, Pr(F) = 1.81×10^{-12}, $p < 0.001$). While other parameters like water depth did change with high tide, many sharks traveled up into HBWC's armored channel or back into the deeper channels in HWBC where depth would not change prey access.

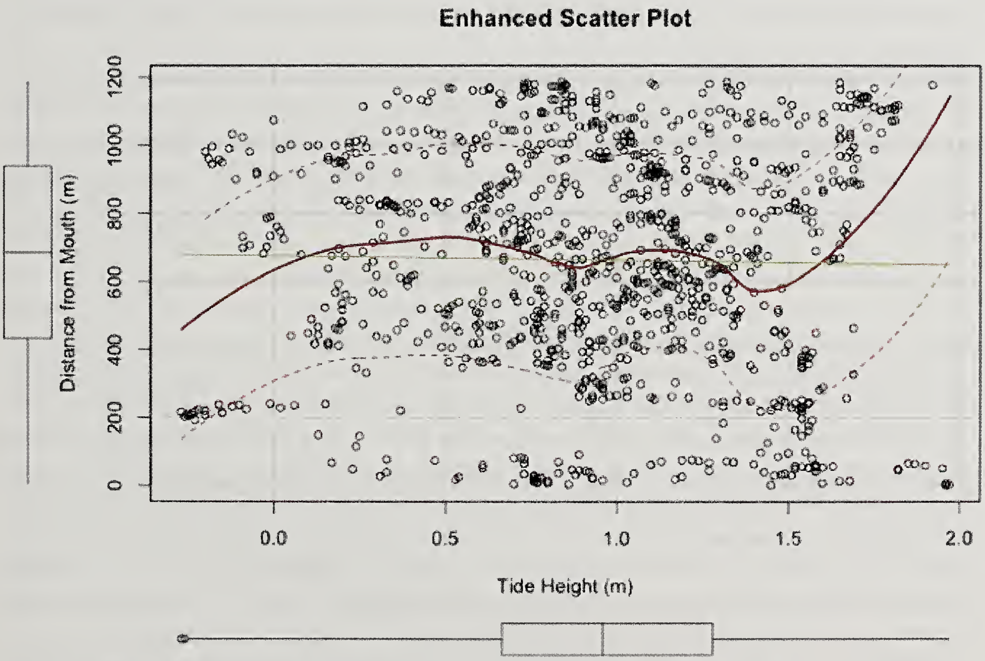

Fig. 2. Relationship between distance from the ocean inlet and tidal height. At the highest incoming tides, *M. californicus* retreat into the inner marshes and channel, likely using those habitats as thermal refuges. Dashed red lines show the confidence intervals, the red line shows the lowess line of best fit and the green line is the linear line of best fit. Box and whisker plots show the quartiles for each set of data.

Discussion

Despite major differences in the amount of available subtidal habitat, individual *Mustelus californicus* used approximately the same amount of area at both sites. Individuals tracked in the HBWC exhibited no difference in 95% and 50% utilization distributions or patch use size compared to those tracked in the BCFTB. They likely limited their movement to similar-sized areas in both sites to regulate energy expenditure (Werner and Hall 1985; Sinervo 1997). Even though HBWC is much narrower, the smaller size does not seem to restrict space use. Its narrow shape does not force *M. californicus* to travel far between microhabitat patches, potentially because eelgrass habitat is relatively continuous along the Channel. While sample sizes were different between BCFTB ($n = 22$) and HBWC ($n = 8$) which may reduce the ability to detect differences, daily activity spaces were less 0.10 km^2 for both 50% and 95% utilization distributions. While the temporal offset of 5 years between the two studies complicates interpretation, the microhabitat amounts and abiotic parameters were collected at the same time within an estuary, allowing us to compare how *M. californicus* select microhabitats.

In addition to using similar habitat sizes, *M. californicus* in both HBWC and BCFTB appear to select areas with eelgrass ecotone, where the edges of eelgrass beds meet the bare soft substratum. Espinoza et al. (2011) found that sharks disproportionately used eelgrass ecotone more, despite its low availability in BCFTB. Sharks in HBWC were located for significantly longer periods in the Channel near the ocean inlet, which was also the habitat with the most eelgrass ecotone available. Eelgrass ecotone is thought to be an important foraging microhabitat for *M. californicus,* as these habitats typically are associated with increased prey density (Kimmer et al. 1998; Leonard et al. 1998; Espinoza et al. 2011; Freedman et al. 2016). The spatial distribution of eelgrass is likely an important driver of habitat selection for this species across all restored estuaries, and could explain why *M. californicus* tend to exhibit high site fidelity to estuaries with abundant eelgrass ecotone.

Temperature appears to be another important driver of *M. californicus* microhabitat selection in both sites; however, the responses to temperature appear to differ by location. We used tidal height as a proxy for water temperature; as incoming ocean water drops the ambient temperatures in the marsh, microhabitats nearest to the ocean inlet are the most affected. Tidal height related significantly to the distance of *M. californicus* individuals from the estuary mouth in both habitats. Espinoza et al. (2011) found that *M. californicus* had core centers of activity in the warmer interior waters of BCFTB (21-23°C), but made forays away from core centers to forage in mudflat microhabitats during cooler high tidal stages. However, distance from estuary mouth does not appear to be related to foraging in HBWC, as individuals swim away from the ocean inlet in the Channel to microhabitats assumed to have high prey density, where armoring excludes tidal mudflats or restored wetland habitat. These forays away from areas of high prey density only occur during cool water periods, which suggests that animals are behaviorally thermoregulating and not foraging. *Mustelus californicus* could also be avoiding larger predators with incoming tide; however, *M. californicus* are often top predators in estuarine systems, as estuaries have shorter trophic structures and larger predators typically do not enter these systems (Able et al. 2004; Allen et al. 2006). Sharks could also use the tidal current as an energy subsidy (i.e. simply moving with the current); however, individuals would typically make movements both against and with current flow during high tide periods to maintain their position in marshes.

In HBWC, *M. californicus* spent the majority of their time in the Channel, the coolest microhabitat within HBWC, likely because high prey densities increase foraging efficiency. Thus, individuals are presented with a trade-off between higher prey density in colder microhabitats and the warmer temperatures that lead to faster growth rates in the back of HWBC's marsh creeks

(Hight and Lowe 2007; Espinoza et al. 2011). When incoming high tides flood the Channel with colder water, the temperatures may fall below *M. californicus'* temperature thresholds, and individuals will likely move into the warmer creek microhabitats as a thermal refuge. Other species of coastal elasmobranchs have shown similar movements between different temperatures to behaviorally thermoregulate (Hight and Lowe 2007; Farrugia et al. 2011). HBWC marsh creeks typically had temperatures closer to what *M. californicus* in BCFTB were found to preferentially use (21°C; Espinoza et al. 2011), while the HBWC Channel has an average temperature of 19°C (Freedman et al. 2016; Whitcraft unpub. data). Even though *M. californicus* seek refuge during times with the lowest temperatures, individuals in HBWC appear to generally tolerate colder temperatures than those in BCFTB to remain in areas with highest abundance of eelgrass ecotone. This suggests that prey densities in the Channel are what drive *M. californicus* to select this habitat over the warmer temperatures available in marsh creeks. The sharks may behaviorally modulate their metabolic rates by moving between foraging grounds and warm water microhabitats. Similar behavioral trade-offs between thermal advantages and food availability have been documented for fishes in laboratory (Wildhaber and Crowder 1990; Krause et al. 1998); and in the field (Garner et al. 1998; Hight & Lowe 2007; Jirik & Lowe 2012).

With these preferred microhabitat conditions in mind, we translocated *M. californicus* to test their site fidelity to channel-type microhabitat. *Mustelus californicus* translocated away from the Channel always returned after translocation, whereas those translocated to the Channel remained there. Translocated individuals consistently returning to the HBWC Channel suggesting that this is a preferred microhabitat for the species. Despite being closer to the thermal range of the preferred microhabitats in BCFTB, HBWC marsh creeks must lack one or more microhabitat conditions that *M. californicus* consider when establishing core activity spaces. The marsh creeks' lack of eelgrass may not support sufficient prey biomass or diversity (Rozas and Minello 1998). However, as eelgrass grows into marsh creeks and the restored habitat and associated communities mature, *M. californicus* may begin to use that microhabitat. Restoration managers have seeded or transplanted eelgrass in newly resorted estuaries to help create high quality microhabitat in their managed sites. In HBWC, eelgrass rapidly expanded shortly after transplantation and it appears to be important to habitat use of target species (Freedman et al. 2016). Future work should try to understand the role of habitat-associated community maturation on fish habitat selection so managers can account for how restored sites may fishes' habitat utilization will shift over time.

Conclusions

To our knowledge, this is the first study to compare the movements of a single fish species in two unique restored estuary designs. Despite the temporal differences between the tracking studies, it is still likely that tagged individuals were responding to available microhabitats in the same ways. Because *M. californicus* have similar habitat utilization areas, habitat size may not be an important factor in driving habitat selection for estuarine fishes after a minimum available subtidal area is met. However, the availability of subtidal warm microhabitats with high prey densities may drive the differences in *M. californicus* abundance seen between BCFTB and HBWC. Espinoza et al. (2011) reported much higher CPUEs than those reported in HBWC (Whitcraft, unpub. data). Large open format tidal basins like BCFTB that probably dampen the temperature change with tidal flux may be better suited to *M. californicus* compared to tidal creek estuaries where animals may have to leave core habitat areas with incoming tides. Additionally, the increased amount of available eelgrass and subtidal foraging area may be an important driver of habitat selection for restoration planners to consider when designing new sites. To design more effective restored estuaries, resource managers must identify target species and create habitats best suited to their needs, as different restoration designs will affect which species benefit most from the planned design. Moving forward, regional managers should focus on creating a diversity of restored estuary designs in the network of estuaries along southern California that should be most effective at supporting a range of juvenile predatory fishes.

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Individually-unique Spot Patterns of Young-of-the-Year Giant Sea Bass (*Stereolepis gigas*) in Captive-raised Fish

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Abstract.—Young-of-the-year Giant Sea Bass (*Stereolepis gigas*) (hereafter YOY GSB) spend the first several months after planktonic settlement within recreational dive limits. After settlement, YOY GSB morph through pigmentation phases where patterns of black spots unique to individual fish appear against the fish's lighter background. In order to prove that underwater photographs of spot patterns could be used to individually identify and possibly track YOY GSB in the field, several YOY GSB were captured and raised at public aquaria. Both sides of each fish were planned to be photographed monthly for a year from the capture date. The black spots of YOY GSB are so few and distinct that computer programs developed to discern individuals of species with complicated spot patterns were not necessary for re-identification of individuals. Three fish that were followed for twelve months in captivity could be individually identified by comparing photographs of their spot patterns by eye. A fourth fish that survived for six months could also be individually distinguished through photographs. This is the first published study to follow the development of YOY GSB spot patterns. Underwater photo-identification techniques could be used to re-identify individuals from several months to at least a year after planktonic settlement. That no capture-recapture studies have been conducted on YOY GSB to date hinders the basic understanding of species ecology and population dynamics. This study opens the door to the use of underwater photography as a passive mark and recapture method for studying YOY GSB along soft-bottomed nursery beaches where they can be found for the first few months after settlement.

The adult Giant Sea Bass (*Stereolepis gigas*) (GSB) is the largest teleost inhabiting California's nearshore habitats, attaining a length of about 2.3 m (7 ft) and a maximum weight of approximately 256 kg (564 lbs) (Baldwin and Keiser 2008). They range from Humboldt Bay, California to Oaxaca, Mexico, including the Gulf of California (Kells, Rocha, and Allen 2016). Adults occur over rocky and sandy bottoms and kelp beds from near shore to approximately 46 m (150 ft) of water (Kells et al. 2016). After their peak commercial catch in 1932 at just over 114,000 kg (12.6 tons), the population quickly crashed and their numbers have remained below historic levels ever since (Pondella and Allen 2008). GSB are now prohibited from intentional take in California by sport and commercial fishermen; however, commercial gill and trammel net fishermen can keep and sell one fish per trip if caught while targeting other species. GSB incidentally caught in other gear, such as squid purse seines, may not be kept.

During the non-spawning season, the background pigmentation of mature GSB is typically gray to dark brown dorsally, fading to a light copper-brown ventrally. Males show a pattern of black spots and white patches throughout their lives. Females exhibit a pattern of black spots and white patches for most of the year, but become dark brown during spawning episodes, totally obscuring the characteristic spots (Hovey 2001).

Young-of-the-year is a term describing a fish less than a year old. YOY GSB occupy habitat between 2 m to at least 38 m (7-125 ft) in depth for the first few months after planktonic settlement. During this period, YOY GSB occupy wide expanses of open sand and mud-bottomed habitat away from rocks, jetties, piers, debris, and other hard structures that often hold predators large enough to eat them at this vulnerable size. YOY GSB pass through several background pigmentation phases and morphological changes during the first few months after settlement, and these transitions help them to appear cryptic while they are hiding to avoid predators (Couffer and Benseman 2015).

When less than 20 mm (0.8 in) in length (all lengths given are total lengths) YOY GSB appear black with several small white patches around the face and sides (Fig. 1). Black-phase YOY GSB have large black dorsal and pelvic fins, and translucent pectoral, anal, and caudal fins. From about 20 mm to about 40 mm (1.6 in), their background pigmentation lightens from black through a brown phase (Fig. 2) into an orange fish (Fig. 3). Dorsal fin pigmentation mirrors the background pigmentation seen on the sides. The large pelvic fins remain black. As the background pigmentation changes from black to brown, the white patches remain, and black spots become visible (Couffer and Benseman 2015).

During the black phase, black spots do not appear to exist on the sides, invisible against the black background. This was discovered when the background pigmentation of a live-captured black-phase YOY GSB that was bottled and placed in a lightless pouch turned nearly white within the space of a few minutes; it returned to black several seconds after re-exposure to sunlight. No black spots were visible during the pigment change, and spots did not appear as the fish returned to black. Unstressed black-phase fish observed during focused surveys at night were initially found black, so it appears that the change in shade within the bottle was a stress-related change, and not a nocturnal change.

In the orange phase, the black spots are distinct against the background. The shapes of these spots may be round, dumbbell-shaped, or square. The sides and both hard and soft dorsal fins can lighten significantly when they become agitated (Fig. 5); however, regardless of the stage of agitation of the fish, all of their black spots remain prominent. Lightening of the background pigmentation enhances the visibility of the black spots, but can obscure some white patches.

By 200 mm (7.9 in), the orange background has become an irregular pattern of bronze and silvery splotches (Fig. 4). Bronze and black pigment has filled the previously-translucent dorsal and anal fins, as well as half of the caudal fin. The pectoral fins are still translucent, and the pelvic fins remain black. The black spots on the sides remain visible, but black spots have also appeared on the dorsal fin. Based on my field observations of 118 individuals during 186 hours of focused surveys to date, YOY GSB of this size have already left the shallow, soft-bottomed nursery areas, and are not easily accessible for study.

Occasionally, YOY GSB in the field displayed damage to their sides that obscured black spots. It is unknown whether or not the black spots that had developed prior to the damage would reappear in the same places on the sides after complete recovery. A full-length caudal fin tear of one captive fish healed so completely in one month that no damage was discernable in photographs. Moderate scrapes and fin tears may be useful for re-identification for a few weeks, but perhaps not over a year's time. However, significant damage might mark a fish for life.

That no capture-recapture studies have been conducted on YOY GSB to date hinders the basic understanding of species ecology and population dynamics of GSB. This is the first published study to follow the development of young-of-the-year GSB spot patterns. Underwater photo-identification techniques could be used as a passive mark and recapture method for studying young-of-the-year Giant Sea Bass along soft-bottomed nursery beaches where they can be found for the first few months after settlement.

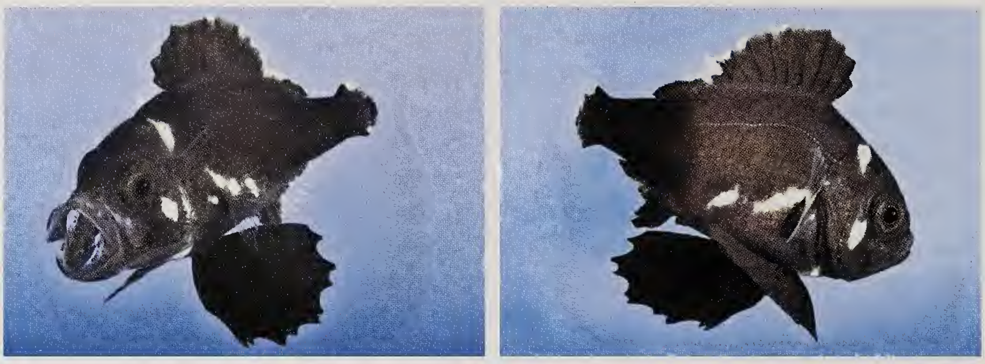

Fig. 1. 18 mm Black-phase GSB.

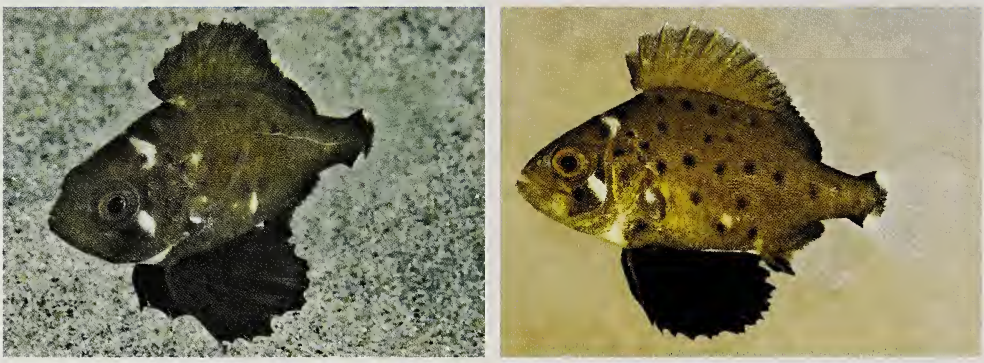

Fig. 2. 30 mm and 37 mm Brown-phase GSB.

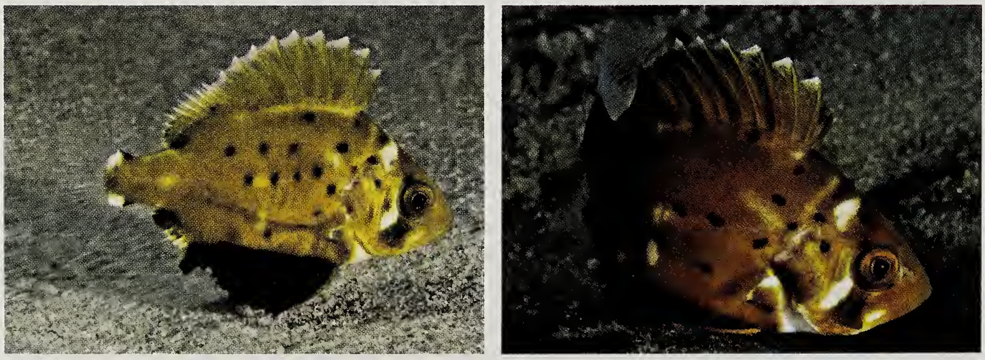

Fig. 3. 40 mm and 45 mm Orange-phase GSB.

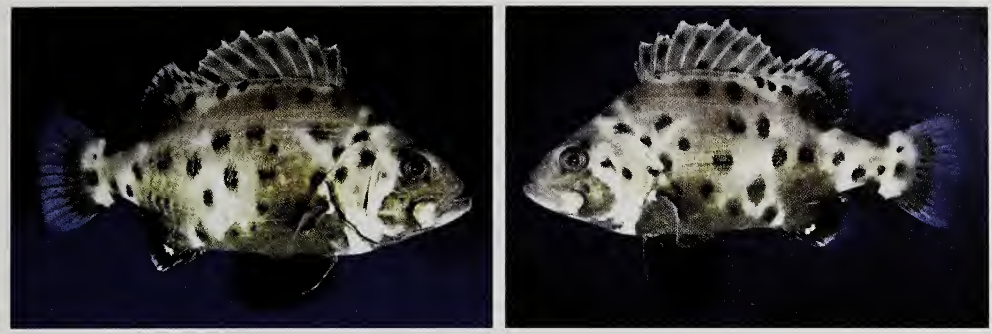

Fig. 4. 194 mm Yearling GSB.

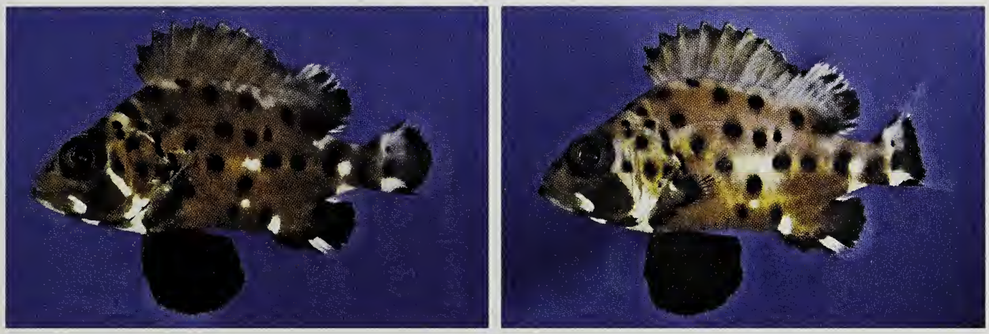

Fig. 5. 98 mm GSB. Background pigment lightens from agitation (shots taken 8 minutes apart).

Materials and Methods

To determine whether or not photo re-identification of individual YOY GSB in the field was possible, I established a baseline by photographically following the changes in spot patterns of captive YOY GSB that were isolated from one another. The main goal of the project was to determine whether or not the black spot pattern of an individual YOY GSB remained similar enough over the course of a year that it might be re-identified using underwater photos taken by recreational divers and focused surveyors in the field. Since all field photos taken by future divers would represent fish of different ages after settlement, it was not necessary that all fish used in the current study be of the same age, size, or pigmentation phase. All YOY GSB followed in this study were captured at different ages after planktonic settlement.

Because YOY GSB husbandry had historically been difficult (Shane et al. 1996), it was possible that not all of the fish being followed for this study would survive a full year in captivity. Collecting photo pairs each month was designed to ensure that if a fish died, any monthly spot pattern changes until death would be documented. Once a month, photos were planned to be taken with the right and left sides of the fish perpendicular to the lens. This would allow a month-to-month comparison of any change in the spot patterns of individual fish, as well as comparisons in patterns between different captive fish.

In 2014, a brown-phase YOY GSB was received by Cabrillo Marine Aquarium in Los Angeles County; I photographed the fish monthly until it expired in 2015. In 2016, my California Department of Fish and Wildlife scientific collecting permit was augmented to include the capture of several YOY GSB. I made verbal agreements with several local aquaria to provide them with fish to raise for the spot pattern study. In return, the aquaria could exhibit the GSB for educational purposes, and could conduct additional studies. I partnered with the Ocean Institute in Dana Point, Orange County, and the Santa Monica Pier Aquarium in Santa Monica, Los Angeles County. As Monterey Bay Aquarium in Monterey County, and Aquarium of the Bay in San Francisco County showed great interest in acquiring YOY GSB for study and educational display, they were also provided with fish, under the condition that they would collect monthly pairs of photos, total lengths, and weights for my spot pattern study.

Many different factors will alter the color of photographs of fish taken in the field and in captivity. In the field, fish colors captured by different underwater photographers are even more difficult to standardize than in aquariums. In addition, all YOY GSB are able to alter their background shade within a few seconds. For these reasons, the specific colors of fish followed in this study were not important. In order to avoid the distraction of differently-pigmented fish and photographic backgrounds, all spot pattern comparison color photos were changed to black and white so that viewers could more easily focus on the spot patterns.

Photographs were taken with digital single lens reflex cameras. A Canon 60 mm lens was used on a cropped-sensor camera body to enhance the depth of field when photographing small YOY GSB. Initially, the two flash heads of a Canon Macro Twin Lite (MT 24 EX) were positioned on either side of the lens using the original equipment mount. As fish grew, the flash heads were spread away from the lens on fabricated arms. A Sigma 35 mm lens was used on a full-sized sensor camera body for larger fish. A rubber lens hood was pressed against the tank to block stray flash reflections from hitting the outside of the glass and entering the lens. Photo backgrounds were placed inside tanks to prevent reflections off the glass aquarium backs. Aquariums purchased for the project were only used for GSB photography to prevent scratches. In order to keep GSB from reacting to their moving reflections off tank bottom glass, tank bottoms were covered with substrate or patterned foam core. The image processing program Adobe Photoshop was used to crop, straighten, and sharpen photos, remove suspended particles, and to alter contrast in order to enhance spot patterns.

The black spots of YOY GSB are so few and distinct that computer programs developed to discern individuals of species with complicated spot patterns were not used for re-identification of individuals. However, a trained eye in the field is not enough to provide proof of re-identification. Clear photographs of the fish's sides provide visual proof of re-identification.

Discussion

I collected 12 pairs of monthly photos of the Santa Monica Pier Aquarium and Ocean Institute fish for one year. This resulted in a series of 24 photos of each fish showing gradual changes in pigment and form as they matured over the course of a year. For brevity, I present these photo pairs at three month intervals for the Santa Monica Pier Aquarium fish (Figs. 6-10), and the Ocean Institute fish (Figs. 11-15). I shot monthly pairs of photos of the Cabrillo Aquarium fish for 6 months until the fish expired (Figs. 16-18); these photo pairs are also presented at three month intervals. The intent of providing YOY GSB to Monterey Bay Aquarium and Aquarium of the Bay was that monthly pairs of side photos would be taken of each fish at the time when morphometric measurements were taken for another study. Unfortunately, two fish died without any monthly side photos being taken, leaving only my initial photos taken the day of capture. I took initial photos of both sides of one YOY GSB before it was transported to Aquarium of the Bay, but the fish died 81 days later without any additional photos of the living fish having been taken. My color image of the left side of the Aquarium of the Bay fish taken the day of capture is shown on the right side of Fig. 2. One of the two Monterey Bay Aquarium fish persisted, and exactly one year after the initial side photos were taken (Fig. 19) I traveled to Monterey to take

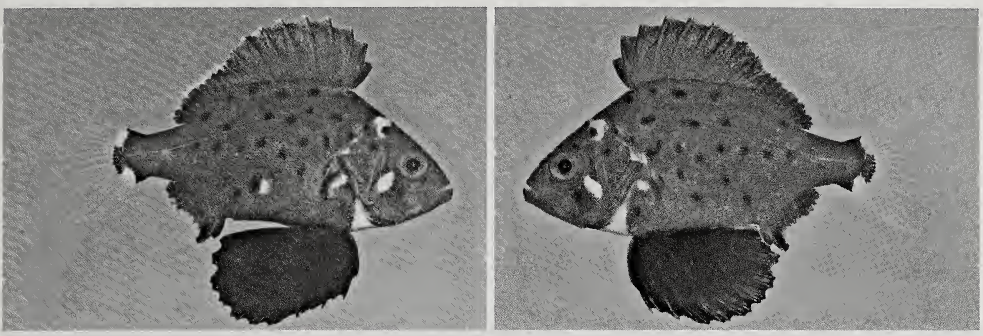

Fig. 6. Capture Date – 27 November 2015 (37 mm).

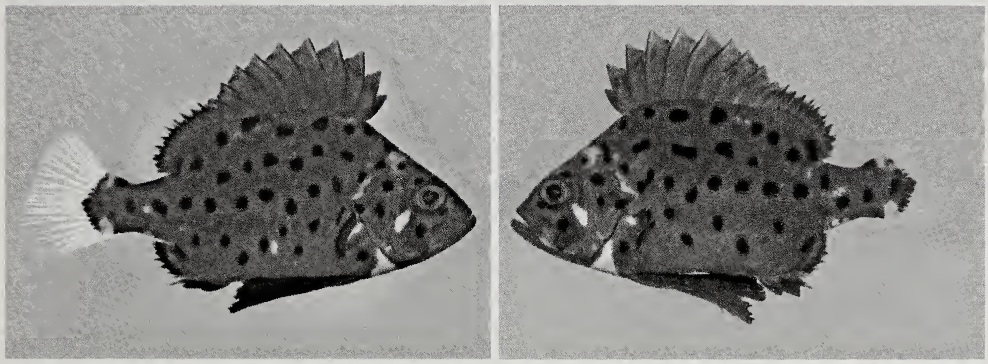

Fig. 7. Three Months from Capture - 16 February 2016 (73 mm).

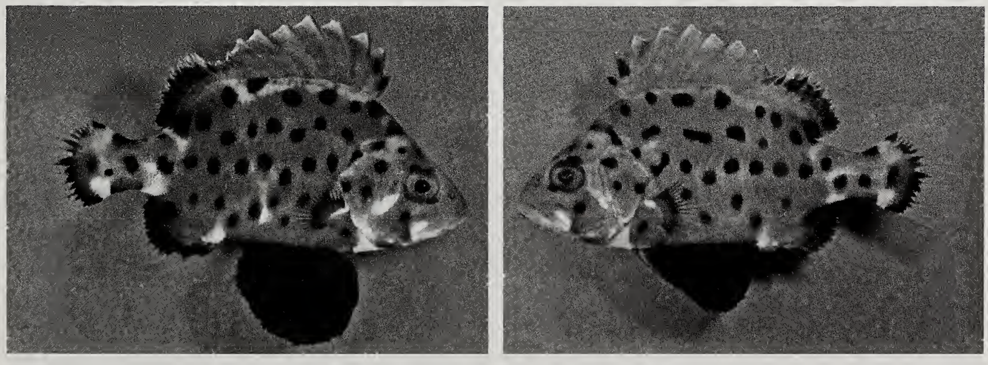

Fig. 8. Six Months from Capture - 14 May 2016 (120 mm).

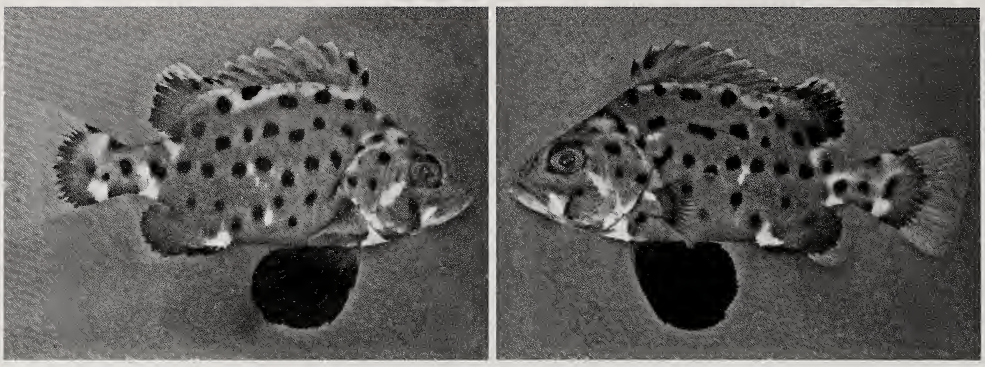

Fig. 9. Nine Months from Capture – 19 August 2016 (146 mm).

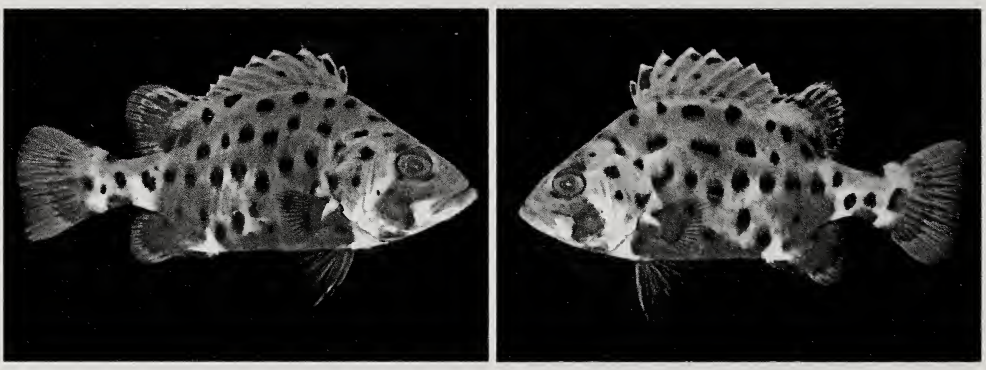

Fig. 10. One Year from Capture - 28 November 2016 (178 mm).

the final photos of this fish (Fig. 20). Although no monthly photos were taken of this fish, the first and final pairs of photos provided evidence for the fish to be included in the study.

Conclusions

The pattern of black spots that develops on the body of a YOY GSB during the transition from the black phase to the brown phase is unique to each individual. Nearly all of the black spots on the sides of the fish become visible during the transition from the black phase to the brown phase. On some fish, a few spots that appear very faint in the brown phase enlarge and darken in the early orange phase. Black spots that develop on the dorsal fin late in the year appear too late in a YOY GSB's development to be used for individual identification during the time when YOY GSB are available to divers within their nursery areas. After all of the black spots on the body become fully visible, their general shapes and relative positions change little across a year's time. This was clearly evident by simple observation of the photographs and did not require sophisticated pattern recognition software to re-identify individuals or to differentiate between individuals.

The black spots of YOY GSB are so few and distinct that computer programs developed to discern individuals of species with complicated spot patterns are not necessary for re-identification of individuals. Each of the three captive-raised YOY GSB that survived for twelve months could be individually identified using photographs of their spot patterns up to a year after collection. Pairs of photos of the Santa Monica Pier Aquarium and Ocean Institute fish taken three months

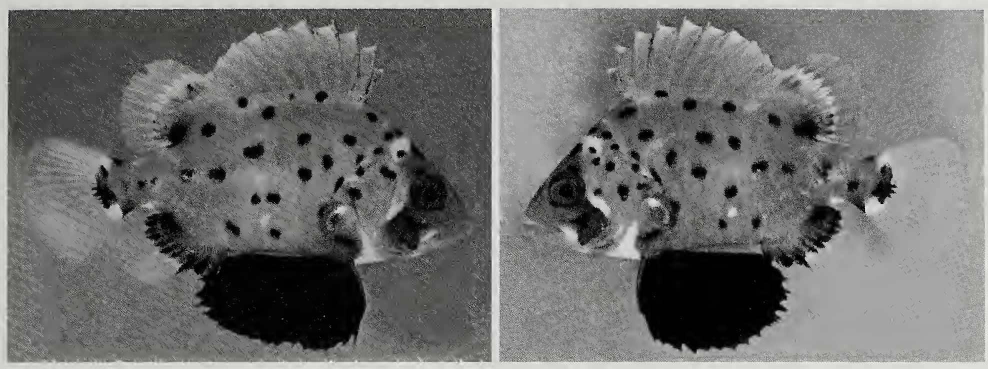

Fig. 11. Capture Date - 19 August 2015 (48 mm).

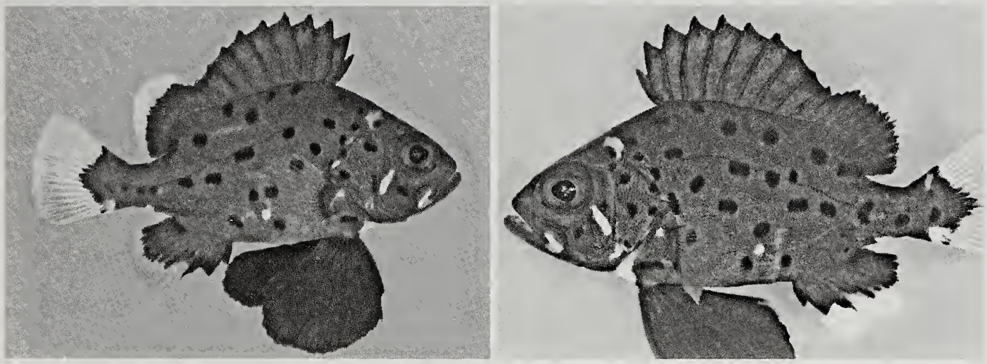

Fig. 12. Three Months from Capture - 17 Nov. 2015 (62 mm on 23 Nov. 2015).

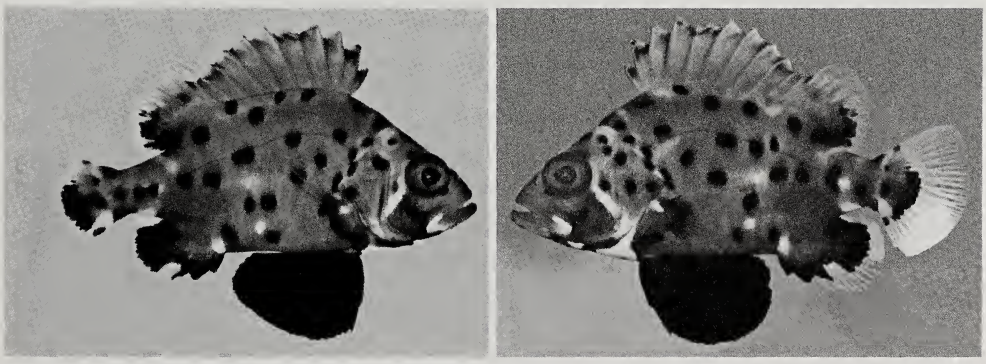

Fig. 13. Six Months from Capture - 18 Feb. 2016 (65 mm on 16 Feb. 2016).

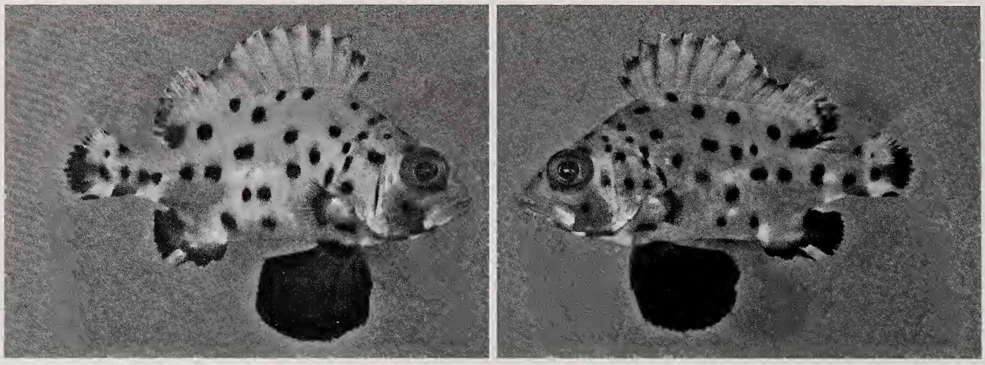

Fig. 14. Nine Months from Capture - 15 May 2016 (90 mm on 18 May 2016).

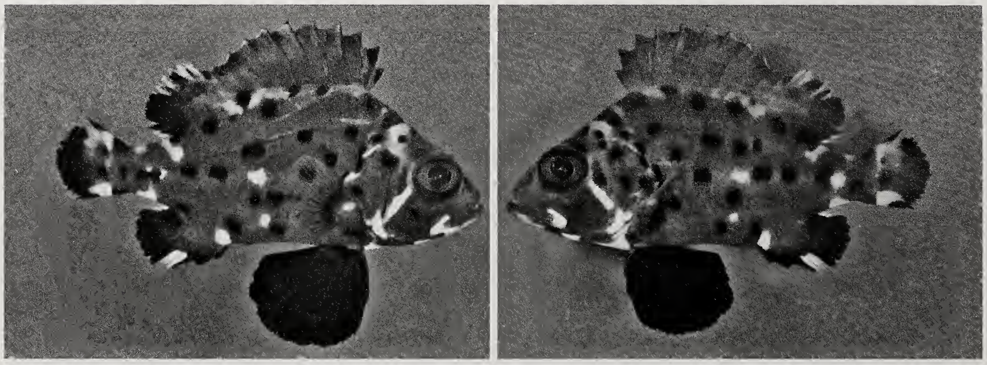

Fig. 15. One Year from Capture - 19 August 2016 (98 mm on 21 August 2016).

apart show a progression of change in spot size, but little change in relative spot positions. Pairs of side photos taken 365 days apart could be compared by eye to identify individuals by their spot patterns. While no intermediate photos were taken of the surviving Monterey Bay Aquarium fish, the initial and final photos, taken 365 days apart, showed that this fish could be re-identified by eye a year later by its spot pattern. The black spot pattern of the Cabrillo Aquarium fish that expired after six months showed little change, and a simple visual comparison of the initial and final photos shows that this fish remained identifiable during its change from brown to orange.

Regularly using the white patches of a black-phase fish to re-identify individuals may be problematic due to the few number of white patches that they present, as well as the difficulty of taking clear side photos of a wild fish that is between 10 and 20 mm in length. However, two photographers diving independently and without knowledge of the other's presence in the area each photographed a black-phase YOY GSB at La Jolla Shores on the same morning. I was present when the first photos were taken, and I measured the fish at 14 mm. That night, I was sent a second set of photos taken by another diver who photographed a fish later in the morning, and identified the second photos as being of the same individual GSB. Although the white patches of black-phase YOY GSB vary in number, size, and placement, it is doubtful that these could regularly be used to re-identify individual black-phase fish; photographic re-identification of individuals is more easily undertaken once the fish turns brown.

This is the first published study following the development of young-of-the-year Giant Sea Bass spot patterns. Underwater photo-identification techniques could be used to re-identify

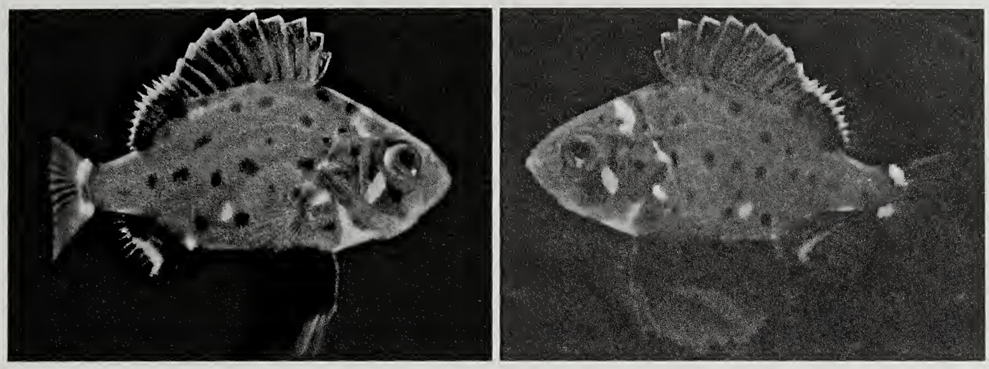

Fig. 16. Initial Photographs – 13 December 2014 (no measurements provided).

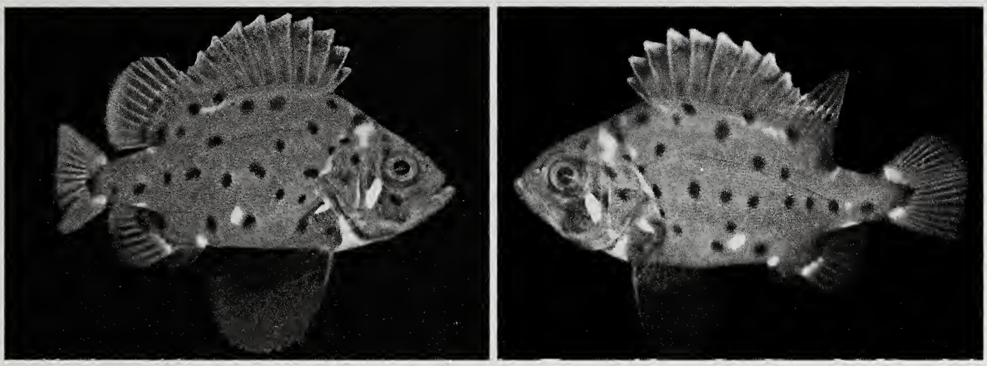

Fig. 17. Two and One Half Months from Capture - 1 March 2015 (no measurements provided).

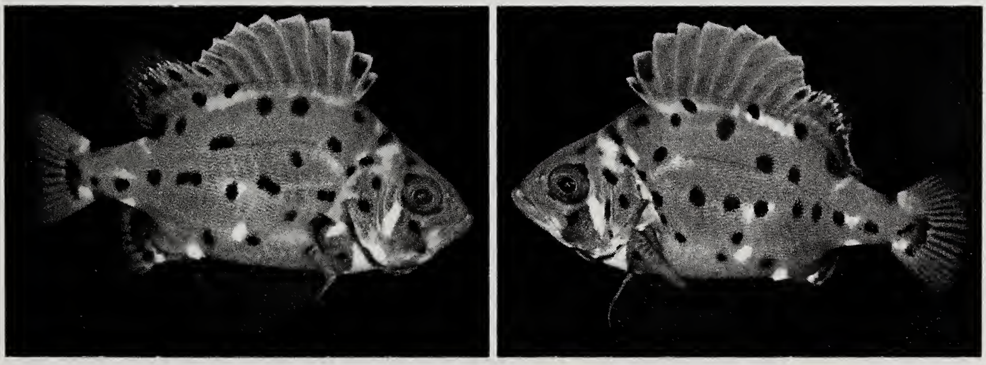

Fig. 18. Six Months from Capture - 17 June 2015 (no measurements provided).

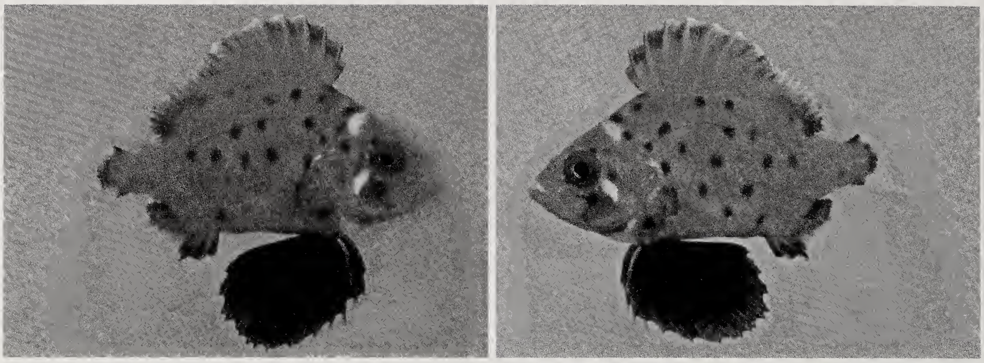

Fig. 19. Initial photo pair by Randy Wilder on 3 September 2015 (48 mm on 11 September 2015).

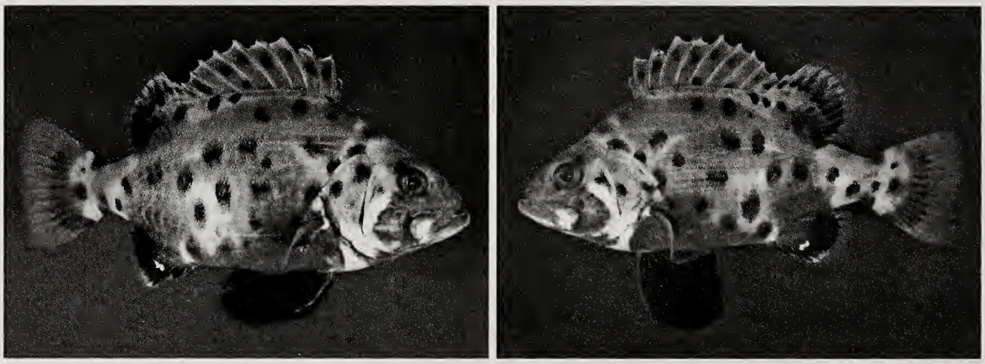

Fig. 20. One Year from Capture (194 mm on 3 September 2016).

individuals from several months to at least a year after planktonic settlement. This study opens the door to the use of underwater photography as a passive mark and recapture method for studying young-of-the-year Giant Sea Bass along soft-bottomed nursery beaches where they can be found for the first few months after settlement.

The few GSB nursery areas that have been located to date lie outside of all of Southern California's Marine Protected Areas, but within one state Marine Conservation Area, where fishing and beach sand replenishment activities are allowed. Details regarding the occupation of these areas by YOY GSB are expected to be published in a Masters thesis by Stephanie A. Benseman of California State University at Northridge. Direct and indirect impacts to these beaches and soft, shallow bottoms immediately offshore such as sedimentation from beach sand replenishment activities during the period when they are occupied by YOY GSB could impact these fish at a very sensitive stage of their development. This proof that individual YOY GSB can be re-identified using photographs of their spot patterns could influence any future protocols or methods developed for YOY GSB presence or absence and preconstruction surveys as well as biological monitoring for construction and maintenance of near-shore structures and beach sand replenishment projects undertaken within currently-known and potentially-occupied YOY GSB habitat.

Acknowledgements

I would like to thank the husbandry managers and staff of the aquaria who invested their time, expertise, resources, and care to raise YOY GSB for this project, and who assisted me with the logistics of tank photography. The surviving fish are available to provide data for other projects. These professionals include Jose Bacallao, Tracey Akino Higa, and Lazaro D. Serrano of Santa Monica Pier Aquarium; Julianne E. Steers, Jessica Brasher, and Kelsey Remmes of Ocean Institute; Kiersten Darrow and the husbandry staff of Cabrillo Marine Aquarium; Kevin O. Lewand, photographer Randy Wilder, and the husbandry staff of Monterey Bay Aquarium. I also thank Stephanie A. Benseman for training me to locate YOY GSB while conducting focused surveys for her Masters thesis at California State University, Northridge. Thanks also to Mark A. Pavelka and Michael W. Mitchell of the United States Fish and Wildlife Service, Larry G. Allen of California State University, Northridge, and several anonymous reviewers for providing valuable editing suggestions on manuscript drafts.

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116(2), 2017, pp. 110–136

A Contribution to the Phylogeography and Anatomy of Helminthoglyptid Land Snails (Pulmonata: Helminthoglyptidae) from the Deserts of Southern California

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Abstract.—Land snails in the family *Helminthoglyptidae* are found sparingly and locally throughout southern California's deserts. They are mostly restricted to rock outcrops and talus in partially shaded canyons where they can gain access to cooler temperatures under the rocks. Several species are known only from their type localities, and were described by shell characters only. We have endeavored to locate known species, document their reproductive anatomy and embryonic shell structure, refine knowledge of their distribution, and incorporate genetic sequencing of two mitochondrial genes (COI and 16S) to investigate evolutionary relationships in these taxa. As a "first pass" molecular study, we have established basic sequence and divergence data for 27 populations of snails in five genera: *Helminthoglypta* (subgenus *Coyote*), *Eremarionta*, *Cahuillus*, *Chamaearionta* and *Sonorelix*. Fifteen of the populations were previously unknown. We confirmed that the Salton Rift/Coachella Valley is a major biogeographic barrier for land snails, as is the north/south transition between the Colorado and Mojave deserts. Described species of *Helminthoglypta* (*Coyote*) grouped together in our phylogenetic analyses and differed from each other by 8-18% in the sequence of the COI gene, concordant with differentiating shell characters. Two previously unknown populations grouped with the *Coyote* species but their COI sequences differed from the described species by 5.7-17% suggesting they may represent undescribed *Coyote* species. Populations of *Sonorelix* from the eastern Mojave were somewhat similar genetically to *Sonorella* spp. from southern Arizona but the precise nature of any relationship between these genera remains unresolved. The remaining, previously unknown populations were genetically close to described species of *Eremarionta*, but inclusion of COI sequences of two *Cahuillus* spp. rendered the genus *Eremarionta* paraphyletic, raising questions about the validity of the names applied to some described species. In particular, the subspecies *E. rowelli bakerensis* was clearly different (>11% in COI) from *E. rowelli amboiana* and *E. rowelli acus*, and deserves elevation to at least species status. The eastern Mojave *Eremarionta* from near Pahrump, Nevada may also be an undescribed species, differing in its COI sequence from its closest described relative by 6.0%. Perhaps the most surprising result from our study was the finding of a population close to the Salton Sea that was very closely related to *E. rowelli* ssp. *bakerensis* which occurs ~200 km further north.

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This highlights the complex nature of genetic variation among geographically isolated *Eremarionta* populations across the eastern Mojave and western Colorado Deserts.

Land snails in the family Helminthoglyptidae Pilsbry, 1939 are sparsely distributed throughout the two contiguous deserts of southeastern California, the Mojave Desert and the more southern and lower elevation Colorado Desert (a subdivision of the Sonoran Desert) (Figs. 1 and 2), as well as in the arid mountain ranges that define their edges. Both deserts are characterized by basin and range topography: rocky, highly eroded arid mountains with lower slopes of gravelly alluvial fans are separated by flat sandy or gravelly expanses. The basins and flats do not provide refugia for snails. For the most part, the mountain slopes do not provide sufficient shelter for snails to survive. It is most often the scattered massive rockpiles and steep, partially shaded canyons with abundant deep talus that provide snail habitat. Topography is thus a major determinant of an extremely patchy distribution of desert snails. Climate is the other determinant, specifically the long drying process of the American Southwest that stretches back at least to the Miocene (Chapin 2008, Mulch et al. 2008). As recently as the Pleistocene, these now arid lands were cooler and moister, vegetated with grassland, chaparral and botanically complex pinyon/juniper woodland interspersed with lakes and rivers (Betancourt et al. 1990, Axelrod 1977). This drying process has isolated previously more widespread populations of snails into narrow canyons, shaded cliff bases and deep talus that provide shelter from desiccation. In these refugia, desert snails spend long periods of time in dormancy between infrequent rain events. Rainfall is concentrated in the winter months as Pacific storms, with summer monsoonal rain occasionally

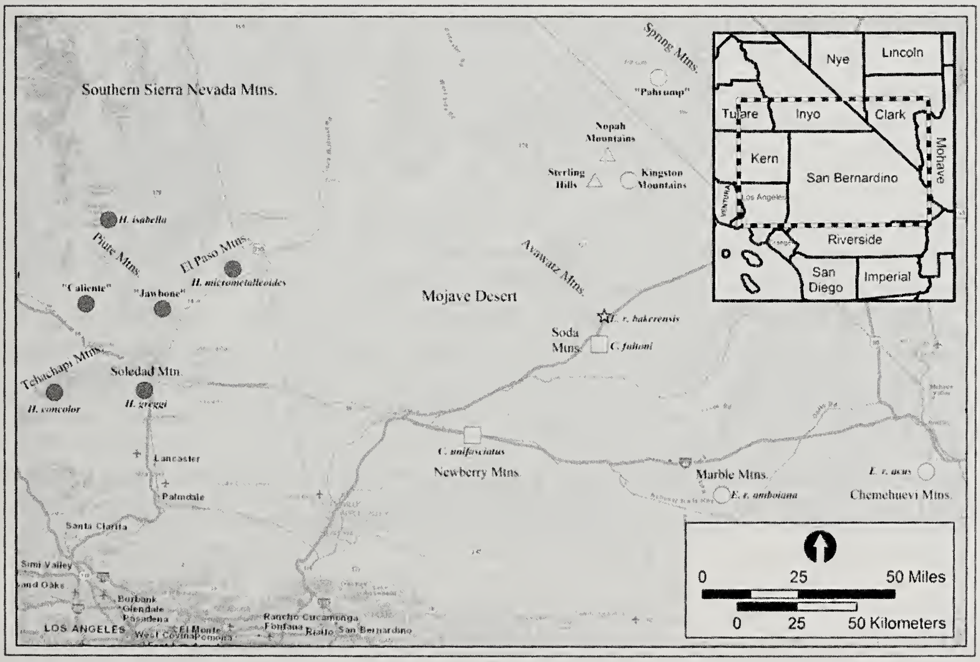
Southern Sierra Nevada Mtns.
H. isabella
Piute Mtns.
El Paso Mtns.
H. micrometalleoides
"Caliente"
"Jawbone"
Tehachapi Mtns.
Soledad Mtn.
H. concolor
H. greggi
Lancaster
Palmdale
Santa Clarita
Simi Valley
Burbank
Glendale
Pasadena
LOS ANGELES
El Monte
West Covina
Pomona
Rancho Cucamonga
Fontana
Rialto
San Bernardino
Mojave Desert
Spring Mtns.
"Pahrump"
Nopah Mountains
Sterling Hills
Kingston Mountains
Avawatz Mtns.
E. r. bakerensis
Soda Mtns.
C. fultoni
C. unifasciatus
Newberry Mtns.
Marble Mtns.
E. r. amboiana
E. r. acus
Chemehuevi Mtns.
Nye
Lincoln
Tulare
Inyo
Clark
Kern
San Bernardino
Mohave
Ventura
Los Angeles
Riverside
San Diego
Imperial
0 25 50 Miles
0 25 50 Kilometers

Fig. 1. Helminthoglypta (Coyote), Sonorelix, and Eremarionta/Cahuillus specimen locations map, Mojave Desert and adjacent mountains. Closed circles: Group 1. Helminthoglypta (Coyote). Triangles: Group 2. Sonorelix. Star: Group 4. *Eremarionta rowelli bakerensis* + Travertine. Open circles: Group 6. East Mojave Eremarionta. Squares: Central Mojave Cahuillus. See Appendices I and II for collection data.

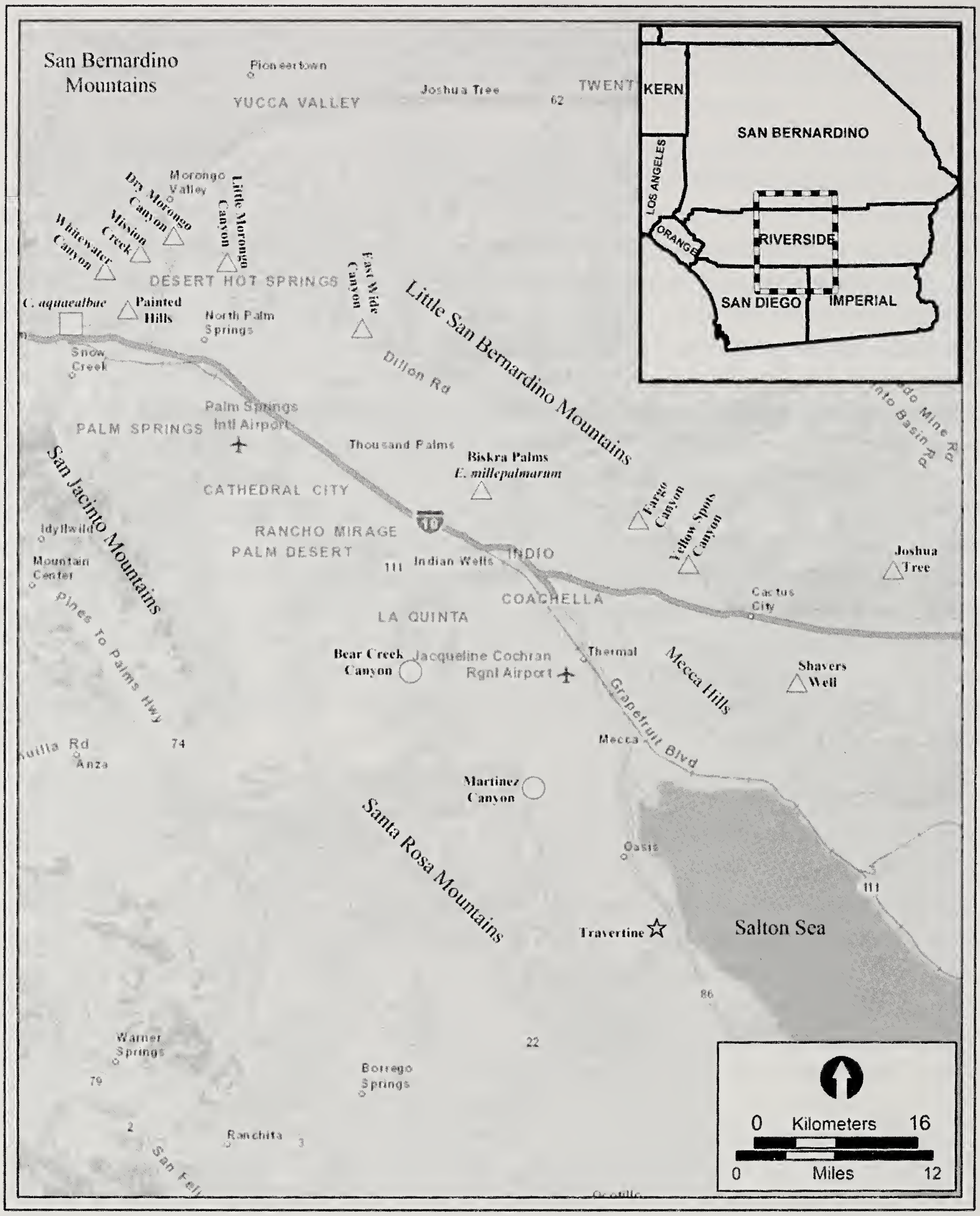
San Bernardino Mountains
Pioneertown
YUCCA VALLEY
Joshua Tree
62
TWENT
KERN
SAN BERNARDINO
LOS ANGELES
ORANGE
RIVERSIDE
SAN DIEGO
IMPERIAL
Morongo Valley
Dry Morongo Canyon
Mission Creek
Whitewater Canyon
Little Morongo Canyon
DESERT HOT SPRINGS
East Wide Canyon
C. aquaealbae
Painted Hills
North Palm Springs
Snow Creek
Little San Bernardino Mountains
Dillon Rd
Palm Springs Intl Airport
PALM SPRINGS
Thousand Palms
Biskra Palms
E. millepalmarum
CATHEDRAL CITY
San Jacinto Mountains
Idyllwild
Mountain Center
10
RANCHO MIRAGE
PALM DESERT
111
Indian Wells
INDIO
Fargo Canyon
Yellow Spots Canyon
Joshua Tree
COACHELLA
Cactus City
LA QUINTA
Pines To Palms Hwy
Bear Creek Canyon
Jacqueline Cochran Rgnl Airport
Thermal
Mecca Hills
Shavers Well
Grapefruit Blvd
Mecca
Anza
74
Martinez Canyon
Santa Rosa Mountains
Oasis
111
Travertine
Salton Sea
86
Warner Springs
22
79
Borrego Springs
2
Ranchita
3
0 Kilometers 16
0 Miles 12

Fig. 2. Chamaearionta, Eremarionta/Cahuillus specimen locations map, Colorado Desert. Square: Group 3. *Chamaearionta aquaealbae*. Circles: Group 5. Colorado Desert Eremarionta/Cahuillus. Star: Group 4. *Eremarionta rowelli bakerensis* + Travertine. Triangles: Group 7. *E. morongoana* + *E. millepalmarum*. See Appendices I and II for collection data.

spilling over into the region from the southeast or south. We can expect varying levels of divergence between the isolated snail populations that have survived to the present. Time from separation, distance between populations, population size, selection pressure and genetic drift should all have had effects on the degree of divergence and potential speciation in the region. Stabilizing selection for ancestral characters and/or convergence have probably contributed to the similarity in appearance of California's desert snails as diverse genetic lines were forced into the same niche, as found in seasonally arid northwestern Australia (Criscione and Köhler 2013, Köhler and Criscione 2015). Convergence on rock-dwelling habits by genetically distant *Xantusia* lizards (which often co-occur with desert snails in Arizona and California) has been noted (Leavitt et al. 2007), resulting in cryptic species.

Most of the desert snail species were described by a handful of scientists in the early 1900s, most notably S. Stillman Berry and George Willett, along with the prolific Henry Augustus Pilsbry. Their pioneering work was, of necessity, based on the snail's morphology, often of the shell only. Variability within and between isolated localities was noted and puzzled over. Later in the 20th century, the baton was picked up by Wendell Gregg, Walter Miller, Barry Roth and others who described additional species and revised the systematics of *Helminthoglypta* and other genera. We have relied heavily on *Checklist of the Land Snails and Slugs of California* (Roth and Sadeghian 2003) as well as the malacology collections at the Santa Barbara Museum of Natural History and the Natural History Museum of Los Angeles County. Recently, malacologists have been able to augment traditional taxonomic methodology with molecular markers (Gilbertson et al. 2013, Roth 2002) though most desert helminthoglyptids have yet to be sequenced.

The purpose of this study is to revisit the type localities of described species and explore new locations to update the taxonomy, anatomy and distribution of California desert snails. To explore divergence and species limits, we provide molecular data on helminthoglyptid snails in the genera *Eremarionta* Pilsbry, 1913, *Cahuillus* Roth, 1996, *Sonorelix* Berry, 1943, *Chamaearionta* Berry, 1930, and *Helminthoglypta* Ancey, 1887, using the mitochondrial genes COI and 16S. We are aware of the limitations of small sample sizes and a reliance on mtDNA only as opposed to the inclusion of nuclear DNA (e.g. Rubinoff and Holland 2005); our intent is to use mtDNA to uncover obvious inconsistencies with taxonomy, to look for cryptic taxa, and to identify groups of snails whose geography and phylogeny will require further, more detailed analysis. We present phylogenetic analyses on combined COI and 16S sequence data, but summarize sequence divergence based on COI alone since the alignment of this locus is typically unambiguous, and to allow for more consistent comparison to other taxa reported in the literature.

Our sampling focused on the western edge and the eastern portion of the Mojave Desert, and the northern and southern edges of the Coachella Valley of the Colorado Desert. See Figs. 1 and 2 for collection locations and Appendices I and II for exact collection data. Our original intent was to confine our collections to desert species, but the Jawbone Canyon *Helminthoglypta* population, despite being at the desert edge, more closely resembled foothill and montane species than the desert "*Coyotes*." Therefore, we obtained samples of the two *Helminthoglypta* subgenus *Coyote* species described from the Piute and Tehachapi Mountains that border the western edge of the Mojave Desert: *Helminthoglypta (Coyote) isabella* Berry, 1938, and *Helminthoglypta (Coyote) concolor* Roth and Hochberg, 1988. We were unable to locate *Helminthoglypta (Coyote) caruthersi* Willett, 1934 from the desert slope of the southern Sierra Nevada, which has not been found since its original description.

Materials and Methods

DNA was extracted from the excised tail tips of individual snails using a DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA) and the manufacturer's protocol. The polymerase chain reaction (PCR) was used to amplify a section of the mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA genes. PCR was conducted in 25 μL volumes containing;

3 μL of DNA template (concentration not determined), 1X ThermoPol PCR Buffer (New England BioLabs, Ipswich, MA), an additional 1 mM $MgCl_2$, 200 μM each dATP, dCTP, dGTP, 400 μM dUTP, 4% (v/v) BSA (NEB), 1.5 U Taq polymerase (NEB), and 0.2 μM of each respective PCR primer. Primers used for COI were LCO1490 and HCO2198 (Folmer et al. 1994), and those used for 16S were 16Scs1 (5′-AAACATACCTTTTGCATAATGG-3′) and 16Sma2 (5′-CTACGGTCCTTTCGTACTA-3′) (Chiba 1999). Reactions were performed in a Mastercycler® ep gradient S thermocycler (Eppendorf North America Inc., New York, NY) with an initial denaturing step of 3 min at 95°C; followed by 38 cycles of 30 s at 94°C, 1 min at 50°C, and 1 min 30 s at 72°C; and, a final extension of 5 min at 72°C. Amplification was confirmed by agarose gel electrophoresis and PCR products were cleaned using the Wizard® PCR Preps DNA purification system (Promega, Madison, WI) and direct-sequenced in both directions at the Institute for Integrative Genome Biology, UCR.

Alignment of forward and reverse reads, and trimming of ambiguous regions from the ends of the consensus sequences, was done using SEQUENCHER 4.9 (Gene Codes Corporation, Ann Arbor, MI). The online tool, EMBOSS Transeq (http://www.ebi.ac.uk/Tools/st/emboss_transeq/) was used to translate the protein coding COI sequence into its amino acid chain, confirming the absence of indels and pseudogenes. Sequences of the COI and 16S genes from closely related and outgroup taxa were retrieved from GenBank (PopSets 451319672 and 451319700 [KC254695-722; Gilbertson et al. 2013]; and, representative sequences of several *Xerocrassa* spp. [FJ627122, FJ627139, FJ627152, JN701868, JN701871, JN701875; Sauer and Hausdorf 2012] and *Sonerella* spp. [COI only; GU344934, GU344936, GU344977, GU345023, GU345038-039; Weaver et al. 2010]). COI sequences were trimmed to match the 580bp sequences of *Sonerella* retrieved from GenBank. All sequences were concatenated, and aligned using MAFFT version 7.050 (http://mafft.cbrc.jp/alignment/software/) with default settings. The resulting matrix contained 66 terminal taxa (including outgroups), each with 1336 nucleotide positions (COI = 580 bp, 16S = 756 bp). Phylogenetic reconstruction was performed by conducting a maximum likelihood (ML) analysis in RAxML (Stamatakis 2006), using the raxmlGUI v. 1.3 (Silvestro and Michelak 2012). The GTR + Γ + I model was applied and the entire dataset was partitioned by locus and, for COI, also by third codon position. Node support was assessed from 10,000 rapid bootstrap replicates as implemented in raxmlGUI (according to Stamatakis et al. 2008). A maximum parsimony (MP) analysis was also conducted in MEGA6 (Tamura et al. 2013). A heuristic search was performed using the Subtree-pruning-Regrafting algorithm (SPR) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). All positions were included in the analysis (gaps treated as missing data) and bootstrap support was assessed with 1000 replicates. Evolutionary divergence was subsequently estimated by calculating average pairwise uncorrected p-distances, based only on COI (due to its unambiguous alignment), among genetic groups identified in the ML and MP analyses (see Results), and among sample locations within those groups, again in MEGA6.

Standard shell measurements were taken as defined in (Arnold 1965). Snails in the upper range of shell diameter for the species or population were determined to be mature and therefore suitable for measuring if 1) live snails possessed a pale, swollen genital pore and/or 2) shell apertures were reflexed and/or 3) terminal growth rugae were crowded, distorted and thickened, with no intervening typical periostracal surface. Reproductive tract measurements are described and defined in association with Figs. 6 and 7. Standard calipers (SPI 2000) were used, often with a string cut to length for measuring curvatures of the organs.

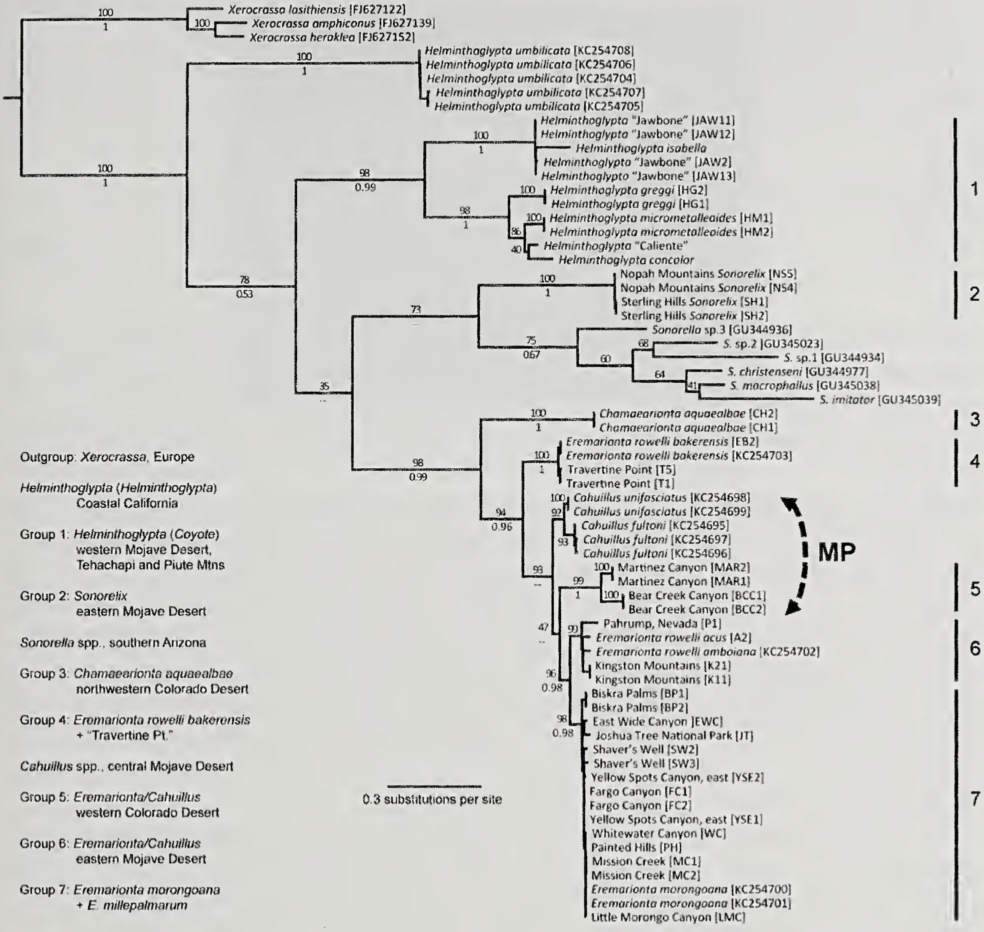
Xerocrassa lasithiensis [FJ627122]
Xerocrassa amphiconus [FJ627139]
Xerocrassa heraklea [FJ627152]
Helminthoglypta umbilicata [KC254708]
Helminthoglypta umbilicata [KC254706]
Helminthoglypta umbilicata [KC254704]
Helminthoglypta umbilicata [KC254707]
Helminthoglypta umbilicata [KC254705]
Helminthoglypta "Jawbone" [JAW11]
Helminthoglypta "Jawbone" [JAW12]
Helminthoglypta isabella
Helminthoglypta "Jawbone" [JAW2]
Helminthoglypta "Jawbone" [JAW13]
Helminthoglypta greggi [HG2]
Helminthoglypta greggi [HG1]
Helminthoglypta micrometalleoides [HM1]
Helminthoglypta micrometalleoides [HM2]
Helminthoglypta "Caliente"
Helminthoglypta concolor
Nopah Mountains Sonorelix [NS5]
Nopah Mountains Sonorelix [NS4]
Sterling Hills Sonorelix [SH1]
Sterling Hills Sonorelix [SH2]
Sonorella sp.3 [GU344936]
S. sp.2 [GU345023]
S. sp.1 [GU344934]
S. christenseni [GU344977]
S. macrophallus [GU345038]
S. imitator [GU345039]
Chamaearionta aquaealbae [CH2]
Chamaearionta aquaealbae [CH1]
Eremarionta rowelli bakerensis [EB2]
Eremarionta rowelli bakerensis [KC254703]
Travertine Point [T5]
Travertine Point [T1]
Cahuillus unifasciatus [KC254698]
Cahuillus unifasciatus [KC254699]
Cahuillus fultoni [KC254695]
Cahuillus fultoni [KC254697]
Cahuillus fultoni [KC254696]
Martinez Canyon [MAR2]
Martinez Canyon [MAR1]
Bear Creek Canyon [BCC1]
Bear Creek Canyon [BCC2]
Pahrump, Nevada [P1]
Eremarionta rowelli acus [A2]
Eremarionta rowelli amboiana [KC254702]
Kingston Mountains [K21]
Kingston Mountains [K11]
Biskra Palms [BP1]
Biskra Palms [BP2]
East Wide Canyon]EWC]
Joshua Tree National Park [JT]
Shaver's Well [SW2]
Shaver's Well [SW3]
Yellow Spots Canyon, east [YSE2]
Fargo Canyon [FC1]
Fargo Canyon [FC2]
Yellow Spots Canyon, east [YSE1]
Whitewater Canyon [WC]
Painted Hills [PH]
Mission Creek [MC1]
Mission Creek [MC2]
Eremarionta morongoana [KC254700]
Eremarionta morongoana [KC254701]
Little Morongo Canyon [LMC]
1
2
3
4
5
6
7
MP
0.3 substitutions per site
Outgroup: Xerocrassa, Europe
Helminthoglypta (Helminthoglypta) Coastal California
Group 1: Helminthoglypta (Coyote) western Mojave Desert, Tehachapi and Piute Mtns
Group 2: Sonorelix eastern Mojave Desert
Sonorella spp., southern Arizona
Group 3: Chamaearionta aquaealbae northwestern Colorado Desert
Group 4: Eremarionta rowelli bakerensis + "Travertine Pt."
Cahuillus spp., central Mojave Desert
Group 5: Eremarionta/Cahuillus western Colorado Desert
Group 6: Eremarionta/Cahuillus eastern Mojave Desert
Group 7: Eremarionta morongoana + E. millepalmarum

Fig. 3. Phylogenetic relationships among southern Californian land snails (Helminthoglyptidae) based on concatenated partial sequences of mtDNA COI and 16S. Maximum likelihood analysis was conducted in RAxML. ML branch support (10,000 bootstrap replicates) is shown above major branches; MP support (1,000 bootstrap replicates) below branches. Dashed arrows indicate alternative placement resulting from maximum parsimony analysis.

Results

Molecular results are presented first, followed by species accounts containing information on comparative morphology, distribution and habitat to provide a baseline characterization of each taxon within the context of the groupings shown in Fig. 3.

There was significant genetic variation across the specimens in our collections. ML analyses grouped these specimens into seven "terminal" groups (at varying evolutionary depth) each with strong support (>92%) (Fig. 3). Group 1 contained all *Helminthoglypta* (*Coyote*) spp.; group 2, all *Sonorelix* spp.; group 3, *Chamaearionta aquaealbae*; group 4, *Eremarionta rowelli* ssp. *bakerensis* + *Eremarionta* from Travertine Point; group 5, *Cahuillus*/*Eremarionta* from the western Colorado Desert; group 6, *Cahuillus*/*Eremarionta* from the eastern Mojave Desert; and, group 7, *Eremarionta morongoana* + *E. millepalmarum*. Phylogenetic relationships among those groups and the ingroup taxa *Helminthoglypta umbilicata* (Pilsbry, 1898) from central coastal California, and members of the genera *Sonorella* from Arizona, and *Cahuillus* from

Table 1. Genetic variation among *Helminthoglypta* subgenus *Coyote* snails (see Group 1, Fig. 3) based on a 580bp section of the COI gene. Mean pairwise uncorrected p-distances calculated using MEGA6.

Taxon	"Jawbone"	*isabella*	*greggi*	*micrometalleoides*	"Caliente"
isabella	0.069				
greggi	0.164	0.163			
micrometalleoides	0.169	0.179	0.116		
"Caliente"	0.168	0.164	0.093	0.057	
concolor	0.170	0.171	0.102	0.078	0.059

the central Mojave (Fig. 3), were generally well resolved with one major exception; a poorly supported branch (35%) grouping *Sonorelix* (group 2) + *Sonorella* and *Chamaearionta* + *Cahuillus*/*Eremarionta* (groups 3-7) as a monophyletic sister group to *H.* (*Coyote*) (group 1). Collapsing this branch results in a topology of *H. umbilicata* as a sister group to a polytomy comprised of *H.* (*Coyote*), *Sonorelix* + *Sonorella*, and *Chamaearionta* + *Cahuillus*/*Eremarionta*. The MP analysis resulted in a single most parsimonious tree (length 2323, r.i. 0.84, c.i. 0.48; results not shown) and recovered the same seven terminal groups each with >98% support and similar ambiguity over the exact relationships between groups 1, 2, and 3-7. However, the sister relationship between *Sonorelix* (group 2) and *Sonorella* was also unresolved creating a four-way polytomy with *H. umbilicata* as a sister group. Group 5 also switched places with the *Cahuillus* group in the MP analysis (alternative positions are indicated by arrows in Fig. 3), but the respective position of these two groups was weakly supported in both analyses (ML = 47%, MP = 72%). In light of the concordant grouping of specimens in the ML and MP analyses, levels of genetic differentiation, and characteristics of shell- and internal morphology are hereafter reported in the context of these seven groups.

Group 1 snails all belong to the subgenus *Coyote* of *Helminthoglypta*. One subgroup of *Coyote* contains *H. isabella* and the "Jawbone" snails. The corresponding subgroup contains *H. greggi* as a basal sister taxon to *H. micrometalleoides*, *H.* "Caliente" and *H. concolor*. Levels of genetic difference in the COI sequence among these six taxa (5.7 – 17.1%; Table 1) lend support to the validity of the named species, two of which were originally described on the basis of shell characteristics alone, *H. isabella* and *H. greggi*.

Group 2 consists of the genus *Sonorelix* and is genetically distant (>16%) from anything else in our sample. We encountered populations of *Sonorelix* in the Nopah Mountains and Sterling Hills in the northeastern Mojave Desert, which appear to be a single species (divergence in COI = 0.3%). ML analyses placed *Sonorelix* as a sister taxon to *Sonorella* species from southern Arizona but that relationship was lost in MP analyses. The genus *Sonorella* was not the focus of this study, but is included in our analysis because it is the most widespread, abundant, and speciose genus in neighboring Arizona (Bequaert and Miller, 1973, Miller and Naranjo-Garcia 1991). Some workers have suggested that *Sonorelix* was derived from *Sonorella* (ibid.), but based on ML and MP analyses of two mitochondrial loci we found little genetic evidence to confirm or refute this hypothesis.

Group 3 consists of the monotypic *Chamaearionta aquaealbae* (Berry, 1922). This species was strongly supported as being sister to the *Cahuillus*/*Eremarionta* complex (groups 4-7) in both ML and MP analyses (Fig. 3). Although genetically related to the *Eremarionta*/*Cahuillus* complex, *Chamaearionta aquaealbae* possesses unique reproductive and shell morphology (embryonic whorls with tightly spaced, apically ascending, elongated papillae (Fig. 4).

The remainder (and majority) of our specimens belonged to the genera *Eremarionta* and *Cahuillus*, two of the dominant snail taxa in the California deserts. Along with a group accessed

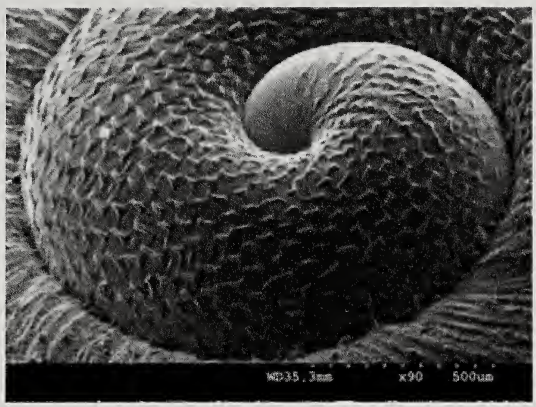

Fig. 4. *Chamaearionta aquaealbae.* SEM of embryonic whorls. SEM specimen LACM 178954.

from GenBank, four groups of *Eremarionta/Cahuillus* were identified (Fig. 3): a group comprising *E. rowelli bakerensis* and specimens from Travertine Point (group 4); a group containing specimens from Martinez and Bear Creek Canyons on the southwestern edge of the Coachella Valley, Colorado Desert (group 5); a group of specimens from the eastern Mojave comprising *Eremarionta rowelli amboiana*, *E. rowelli acus*, and previously unknown populations near to Pahrump and the Kingston Mountains (group 6); a group comprising specimens of *E. morongoana* and *E. millepalmarum* from the northern edge of the Coachella Valley (group 7); and a *Cahuillus* group comprising *C. unifasciatus* and *C. fultoni* from the central and eastern Mojave Desert (sequences from GenBank). While the *Cahuillus* group and groups 5-7 are individually well-supported, the relationships between these groups remains unresolved in the ML analysis, with only 47% support (Fig. 3). See Table 2 for COI uncorrected p-distances for groups 4-7.

Group 4 contained *Eremarionta rowelli bakerensis* (Pilsbry and Lowe, 1934), known only from the hills behind the town of Baker in the Mojave Desert. Thus, it was surprising to find a very close genetic relative 250 km away in the Colorado Desert, near Travertine Rock, at the

Table 2. Genetic variation among *Eremarionta/Cahuillus* species/populations from the Mojave and Colorado Deserts of southern California based on a 580bp section of the COI gene (see Groups 4-7, Fig. 3). Mean pairwise uncorrected p-distances calculated using MEGA6. Shaded values are of Group 6 snails. LMC (Little Morongo Canyon) is included as an example from Group 7.

Taxon (Group)	TR (4)	*bakerensis* (4)	*C. unifasciatus*	*C. fultoni*	MAR (5)	BCC (5)	LMC (7)	*acus* (6)	KI (6)	P (6)
bakerensis (4)	0.007									
Cahuillus unifasciatus	0.119	0.116								
Cahuillus fultoni	0.122	0.121	0.044							
MAR (5)	0.122	0.122	0.097	0.106						
BCC (5)	0.139	0.136	0.109	0.124	0.062					
LMC (7)	0.126	0.122	0.071	0.094	0.093	0.107				
acus (6)	0.117	0.114	0.083	0.097	0.091	0.102	0.064			
KI (6)	0.119	0.119	0.086	0.094	0.086	0.102	0.064	0.033		
P (6)	0.133	0.131	0.098	0.110	0.103	0.116	0.079	0.059	0.055	
amboiana (6)	0.126	0.124	0.086	0.094	0.084	0.109	0.062	0.029	0.026	0.060

Table 3. Genetic variation among Group 7 (*Eremarionta morongoana* and *E. millepalmarum*) populations from the northern edge of the Coachella Valley of Southern California based on a 580bp section of the COI gene. Mean pairwise uncorrected p-distances calculated using MEGA6. Populations listed west to east. WC = Whitewater Canyon; PH = Painted Hills; MC = Mission Creek; LMC = Little Morongo Canyon; EWC = East Wide Canyon; BP = Biskra Palms; FC = Fargo Canyon; YS = Yellow Spots Canyon; SW = Shaver's Well; JT = Joshua Tree National Park

Taxon (Group)	WC	PH	MC	LMC	EWC	BP	FC	YS	SW
PH	0.002								
MC	0	0							
LMC	0.002	0	0						
EWC	0.020	0.019	0.019	0.019					
BP*	0.023	0.022	0.022	0.022	0.022				
FC	0.008	0.007	0.007	0.007	0.016	0.019			
YS	0.010	0.008	0.008	0.008	0.017	0.020	0.005		
SW	0.019	0.018	0.018	0.018	0.024	0.024	0.015	0.016	
JT	0.029	0.028	0.028	0.028	0.021	0.033	0.026	0.025	0.036

* BP (Biskra Palms) = *Eremarionta millepalmarum*.

southwestern edge of the Coachella Valley (Fig. 2). COI sequences of *E. rowelli bakerensis* and the Travertine population (T) differed by only 0.7% (well within typical interspecific boundaries) and the latter is likely a disjunct population of *E. rowelli bakerensis*. By comparison, *E. rowelli bakerensis* differed from two other subspecies of *E. rowelli* included in our study by >11% (Table 2). The *Cahuillus* species from the central Mojave (accessed from GenBank) formed a monophyletic group, genetically akin to *Eremarionta*.

Group 5 consisted of two populations of *Eremarionta/Cahuillus* sampled from the western edge of the Coachella Valley at the base of the Santa Rosa Mountains: MAR = Martinez Canyon and BCC = Bear Creek Canyon (Fig. 2). These populations are expected to be closely related to *Cahuillus indioensis* (Yates, 1890), a species which is currently divided into several subspecies found along the base of the Santa Rosa and San Jacinto Mountains to the north of our collections (Roth and Sadeghian 2003). *Cahuillus indioensis* is currently under revision by LHG, DMG, and others. Genetically, the MAR population differs from that of BCC by 6.2%, yet geographically they are separated by only 16.5 km. Neither location had been sampled previously, and the specific identity of both remains unresolved.

Group 6 comprised DNA sequences of samples from three eastern Mojave Desert locations (Fig. 1): Needles (A), the Kingston Mountains (KI), and near Pahrump, Nevada (P), which grouped with *Eremarionta rowelli amboiana* from GenBank (KC254702). The Needles population is referable to *Eremarionta rowelli acus* (Pilsbry, 1939) based on geographic range, but the remaining two populations are undetermined. We currently lack detailed morphological information on the Pahrump taxon, with only one juvenile available for study. Levels of genetic difference within this group ranged from 2.6% to 6.0% (Table 2), but given the relative distances between current sample locations, defining true species boundaries will likely require a much greater sampling effort. That said, it appears that members of this group are the dominant helminthoglyptids in the eastern Mojave Desert (Fig. 1).

With the exception of *Chamaearionta aquaealbae*, all other populations from the northern and eastern edges of the Coachella Valley grouped together with sequences of *Eremarionta morongoana* from GenBank (KC254700-701) to form a final group; group 7 (Figs. 2 and 3). Genetic distances (COI) are shown in Table 3. A somewhat genetically divergent population (2.1-3.6% in COI) from the southern edge of Joshua Tree National Park (JT) also fell into this group, which was strongly supported (>98%) in both ML and MP analyses. Another eastern population, Biskra Palms (BP), is referable to *Eremarionta millepalmarum* (Berry, 1930). Biskra Palms is the second most divergent population (1.9-3.3% in COI) but this level of genetic difference may not be sufficient to confirm that this should be treated as a different species. Genetic variation among other populations in this group peaked at 2.4% (Table 3) suggesting they are all *E. morongoana*. The Shavers Well population (SW) was originally described as *Eremarionta brunnea* (Willett 1935), but it is poorly differentiated from the other Group 7 snails. The shell morphology of snails from populations to the east of Little Morongo Canyon (LMC; Fig. 2) was not typical of those from the type locality of *E. morongoana* in Dry Morongo Canyon, and populations to the west of there. This is clearly a group that would benefit from further molecular and morphological work, perhaps leading to taxonomic revision.

Group 1, *Helminthoglypta* (*Coyote*) spp. All of the desert *Helminthoglypta* belong to the subgenus *Coyote* Reeder and Roth, 1988. This subgenus was described by Reeder and Roth, (1988) based on "a prominent bulge at the anterior end of the upper, double-tubed chamber of the penis", and a flattened, papillose shell. It is exclusively southern Californian in distribution. The type species is *Helminthoglypta (Coyote) taylori*, a narrow endemic from the desert foothills of the San Bernardino Mountains (ibid.). We sampled the type localities for *Helminthoglypta* (*Coyote*) *greggi* Willett, 1931 and *Helminthoglypta (Coyote) micrometalleoides* Miller, 1970. We were unable to obtain live specimens of *H. micrometalleoides* from Red Mountain, the only other recorded location for this species.

In group 1, two unassigned populations, "Caliente" and "Jawbone", are poorly differentiated from other group 1 snails. Shell dimensions of subgenus *Coyote* snails (Table 4) were subjected to statistical analysis in an attempt to corroborate the molecular differentiation. The "Caliente" population was excluded due to small sample size. A discriminant analysis of shell morphometrics of Group 1 snails successfully identified taxa based primarily on two functions. The first function represents a composite measure of shell size (expansion rate, diameter, height, aperture height, and aperture width), while the second is primarily based upon whorl count. Fig. 5 illustrates the nature of the differences based upon these two functions (general shell size and whorl count) for the five groups. *Helminthoglypta greggi* and *H. micrometalleoides* were readily distinguishable by the functions (Fig. 5). *Helminthoglypta isabella, H. concolor,* and the Jawbone Canyon snail have similar dimensions, and their composite functions could correctly place only a portion of their shells in the correct taxon (Table 5); 75% and 70% respectively of the Jawbone Canyon and *H. isabella* snails were correctly identified. As seen in Fig. 5 the Jawbone Canyon snail was slightly smaller than the *H. concolor* snails but larger than the *H. isabella* snails. Though larger in size, *H. concolor* did not separate cleanly in the discriminant analysis with only 50% placed correctly. This analysis should benefit from increasing the sample size.

Helminthoglypta greggi (Fig. 6): All museum records of *H. greggi* are from Soledad Mountain in Kern County. We located *H. greggi* on only two other hills near to the type locality: Standard Hill, 3 km northeast of Soledad Mtn., and the other 6 km to the west at Middle Buttes. Both hills have been extensively mined, with very little undisturbed habitat remaining. The single live snail found at Middle Buttes genetically groups closely with the Soledad Mountain snails (D. Eernisse, unpublished data). The only evidence from Standard Hill is a single shell, morphologically very similar to Soledad specimens. We repeatedly searched other nearby hills, particularly those of comparable height and area to Soledad Mountain such as Tropico and Rosamond Hills without finding any snails. *H. greggi* therefore appears to be restricted mainly to Soledad Mountain, with small populations on the two other hills mentioned above. Soledad Mountain has been and still is being extensively mined, with serious reductions in undisturbed

Table 4. Shell Measurements of *Helminthoglypta* (*Coyote*) taxa. Upper value: mean, lower value: standard deviation. Valid N listed under species name. Measurements in millimeters.

Taxon	Whorl count WC	Greater diameter GD	Height HT	Aperture height AHT	Aperture width AW	Umbilicus diameter UD	Expansion rate GD/WC
"Jawbone" (12)	**5.06** (.138)	**20.02** (1.101)	**11.02** (.818)	**10.31** (.597)	**10.37** (.935)	**3.20** (.335)	**3.96** (.184)
isabella (10)	**5.29** (.137)	**20.51** (1.813)	**11.76** (.711)	**10.95** (1.074)	**9.87** (1.008)	**3.54** (.493)	**3.87** (.276)
greggi (11)	**5.05** (.144)	**14.15** (.545)	**7.45** (.759)	**6.24** (.361)	**6.23** (.450)	**2.73** (.294)	**2.81** (.075)
micrometalleoides (5)	**4.6** (.144)	**11.94** (.568)	**6.30** (.752)	**5.46** (.670)	**5.34** (.272)	**2.73** (.179)	**2.60** (.173)
concolor (6)	**5.23** (.137)	**21.43** (1.081)	**11.60** (.759)	**10.45** (1.34)	**10.62** (.776)	**3.40** (.400)	**4.11** (.173)

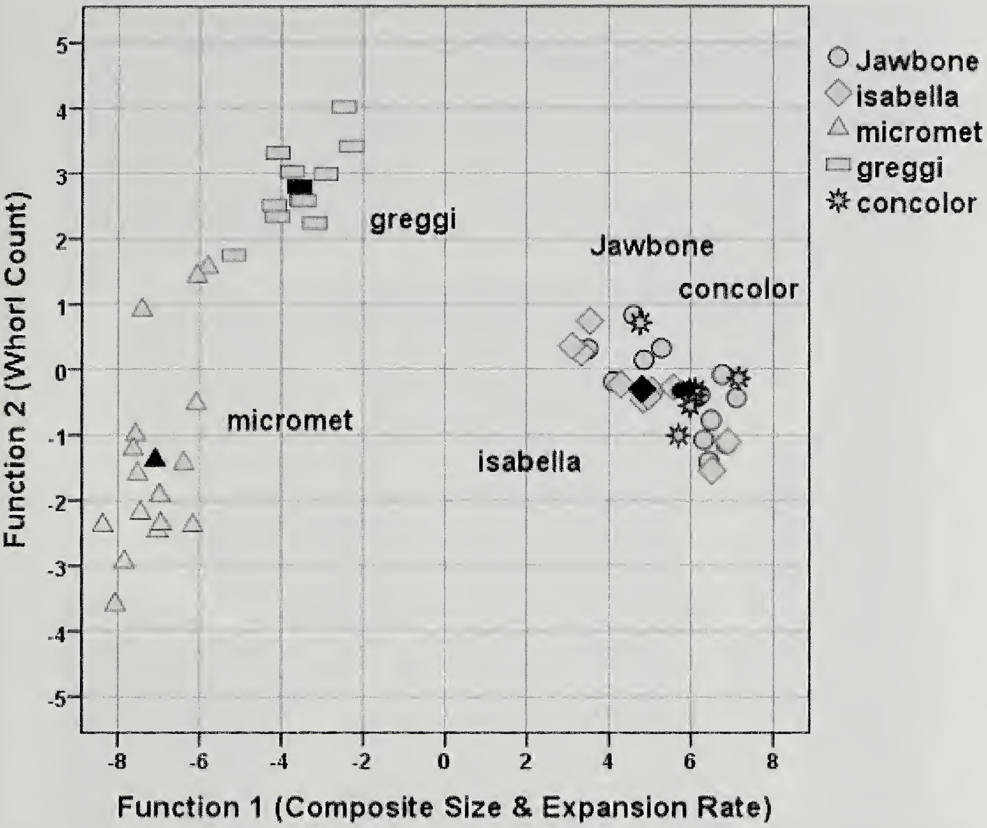

Fig. 5. Canonical Discriminant Function comparison of 5 populations of *Helminthoglypta* (*Coyote*) (Group 1). Filled symbols are the group centroids. micromet = *H. micrometalleoides.*

habitat. *H. greggi* is found in igneous rock outcrops and talus on slopes of low sparse desert scrub.

The species most similar to *H. greggi* in appearance, habitat, and type of location (small isolated ranges near the western edge of the Mojave Desert) is *H. micrometalleoides*. All our collections were from the type locality as described by Miller (1970) in Iron Canyon, in the northern El Paso Mountains. We did not acquire live samples from the only other known population of this species at nearby Red Mountain. Despite the morphological and ecological

Table 5. Predicted group membership of *Helminthoglypta* (*Coyote*) taxa by discriminant functions (Number of specimens assigned to each taxon).

Taxon	"Jawbone"	isabella	micromet*	greggi	Concolor
"Jawbone"	9	2	0	0	1
isabella	1	7	0	0	2
micromet*	0	0	5	11	0
greggi	0	0	0	0	0
concolor	2	1	0	0	3

* micromet = micrometalleoides.

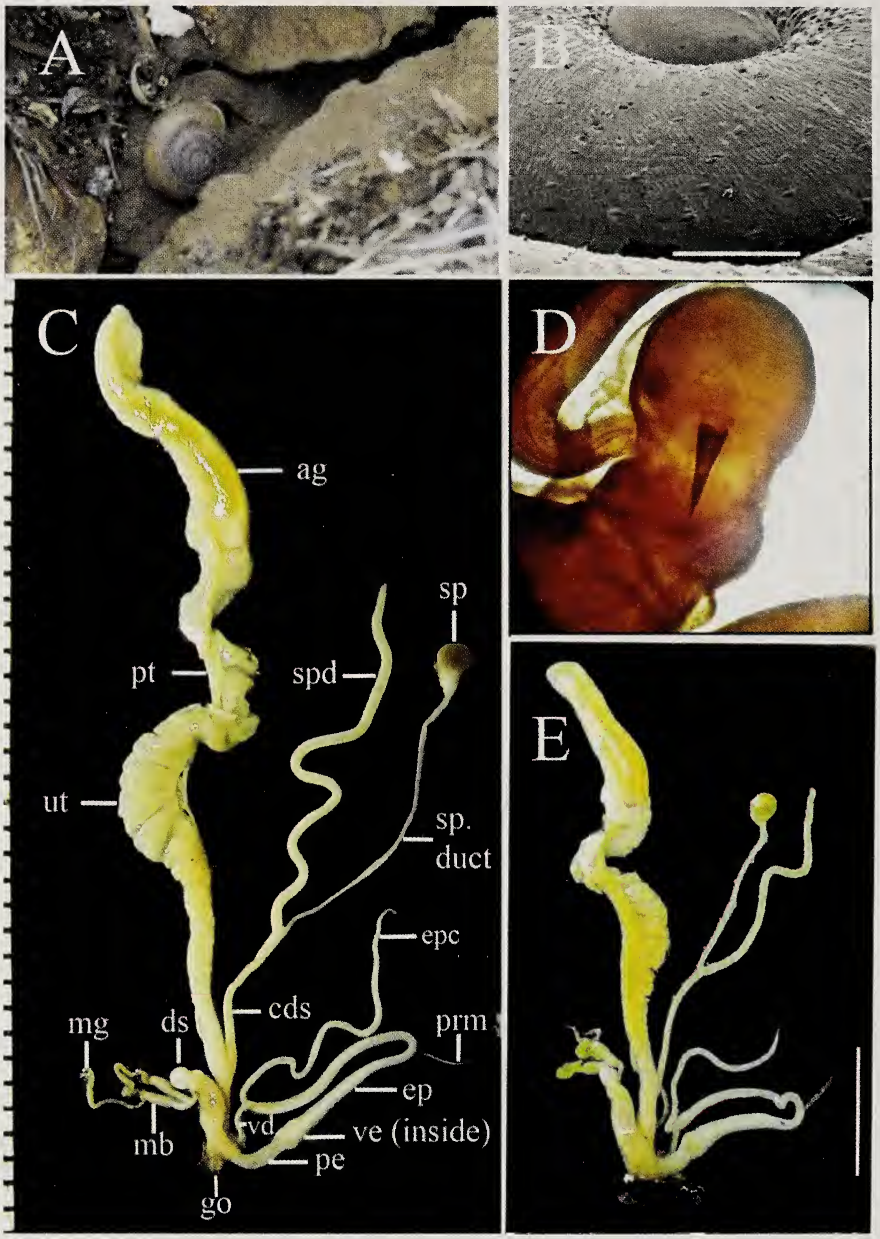

Fig. 6. A. *Helminthoglypta* (*Coyote*) *greggi*, photograph of the snail *in situ*. B. SEM of embryonic whorls of *H. greggi* (in part). Scale = 300um. C. Freshly dissected reproductive tract of *H. greggi*. Scale in mm. Measurements: Table 4. D. Stained *H. greggi* dart sac with dart. Magnification = 40X. E. Reproductive tract of *H. micrometalleoides* (at same scale as *H. greggi*). Scale bar = 5 mm. Anatomical and SEM specimens: LACM 178952 (*H. greggi*) and LACM 178953 (*H. micrometalleoides*). Abbreviations: ag, albumen gland; ds, dart sac; ep, epiphallus; go, genital orifice; mb, mucus bulb; mg, mucus gland; prm, penial retractor muscle; pt, prostate gland; ut, uterus; vd, vas deferens; ve, verge; others as in Table 6.

Table 6. Comparative reproductive morphology measurements: *Helminthoglypta* (*Coyote*) *greggi* and *Helminthoglypta* (*Coyote*) *micrometalleoides* (fresh, illustrated specimens) and *Helminthoglypta* (*Coyote*) *concolor* (slide-mounted specimen, Roth and Hochberg 1988). Measurements are to the closest 0.5 mm. Abbreviations: CDS, common duct of the spermatheca (copulatory canal); SPDU, spermathecal duct (bursa duct); SP, spermatheca (bursa copulatrix); TOT, total length of CDS + SPDU + SP; SPD, spermathecal diverticulum (bursa tract diverticulum); VA, vagina; FO, free oviduct; EPC, epiphallic cecum; SWEP, single-walled section of epiphallus (proximal region); DWEP, double-walled section of epiphallus including verge (distal region); PE, penis. Micro = micrometalleoides.

Species	CDS	SPDU	SP	TOT	SPD	VA	FO	EPC	SWEP	DWEP	PE
greggi	6.0	12.5	1.5	20.0	17.0	2.5	2.0	13.0	10.0	4.0	3.0
micro-	5.5	6.0	1.0	12.5	10.0	2.0	2.0	7.0	8.0	3.0	2.0
concolor	13.0	16.0	2.0	31.0	33.5	3.5	6.0	38.5	17.0	6.5	4.0

similarities between these two species, they are not each other's closest relatives and genetic divergence between the two species was 11.6% (Table 1).

The organs of the reproductive tract of *H. greggi* are noticeably longer overall than the organs of the smaller *H. micrometalloides* (Table 6, Fig. 6C and E). This is especially noted for the lengths of the spermathecal duct and diverticulum, as well as the epiphallic cecum. Secondly, its epiphallus is more elongated and cylindrical (i.e. not bulging as noticeably in the distal region). The mucus gland bulbs of *H. greggi* are elongated as well. Otherwise, the reproductive tracts of these two species show similar characteristics and are typical of subgenus *Coyote*.

The SEM of the embryonic whorls of *Helminthoglypta greggi* shows ornamentation with rounded elongated papillae arranged in apically ascending, well-spaced, rather ill-defined, spiral rows separated by cross-rows of numerous short rugae. (Fig. 6-B). Both the papillae and rugae are much smoother (abraded-looking) than typical for helminthoglyptids. However, the shell was fresh and immature (10.2 mm diameter) and should not have been subject to much, if any, obvious abrasion. Shell coloration in adults is tan to light brown, and body color is black fading to beige on the foot.

Helminthoglypta concolor was previously known only from the type locality, where it was found under fallen bark and logs of White Fir (*Abies concolor* (Gordon & Glend.) Lindley) (Roth and Hochberg 1988). We have expanded the known range to two additional canyons in the Tehachapi Mountains, Cottonwood and El Paso canyons, the latter being 12 km southwest of the type location in Tejon Canyon. These snails are found on north-facing conifer or mixed oak/conifer woodland slopes high on the coastal side of the Tehachapi Mountains. Elevations are higher than for any of the other *Coyote* taxa in this paper, between 1,623 and 1,803 m (5,325 – 5,912 ft). Swaths of suitable habitat are separated from each other by large tracts of chaparral and oak woodland savannah. These large, dark brown snails have rugose shells with more frequent and prominent papillae compared to *Helminthoglypta greggi* and *H. micrometalleoides*. The mantle as seen through the shell is irregularly mottled with dark spots and patches, unlike the two desert species which appear uniformly light brown with an indistinct shoulder band.

Helminthoglypta isabella is known only from the type locality, which was rather vaguely described by the collector (Berry 1938). The species still persists at the type vicinity south of Isabella Reservoir, and appears to be most abundant around the town of South Lake at a series of limestone outcrops in a predominantly granitic region. Elevations of three localities range from 939 to 1016 m (3,081-3,333 ft). It was described as being found underneath dead clumps of *Hesperoyucca whipplei* (Torr.) Baker, but it is also found under rocks and in rock crevices.

The habitat of *H. isabella* is dry rocky slopes vegetated with open chaparral with scattered oaks and pines. Its reproductive morphology is unknown.

The previously unknown "Jawbone" snail grouped closely with *H. isabella* but still differed by 6.9% in COI, and it is unclear whether or not it represents an undescribed species (Fig. 3). Shell analysis was similarly suggestive but inconclusive (Table 5, Fig. 5). Its shell is similar in appearance to that of *H. isabella* but paler in color. The "Jawbone" snail is found in narrow side canyons and talus in desert scrub in the lower portion of Jawbone Canyon, at elevations ranging from 881-922 m (2,890-3,025 ft). Indicator plants for suitable habitat are Bladdersage (*Scutellaria mexicana* (Torr.) A. J. Paton and Mormon Tea (*Ephedra nevadensis* S. Watson when suitable rock features are also present. It is a narrow endemic, with a total range of about 32 km^2, about 44 km southeast of *H. isabella* (Fig. 1).

The specific status of population "Caliente" is also unresolved. Genetically, "Caliente" is closely related to *H. concolor* and *H. micrometalleoides* (Fig. 3, Table 1), but characterizing the true relationship between these three taxa will likely require additional samples. To date, it has been found only in the steepest and shadiest portion of Caliente Canyon on the west side of the Piute Mountains, in mixed oak/Gray Pine (*Pinus sabiniana* Dougl.)/Buckeye (*Aesculus californica* (Spach) Nutt.) woodland at approximately 700 m (2,300 ft) in elevation. Caliente Canyon runs through the southwestern slopes of the Piute Mountains, about 32 km. north of the type locality for *H. concolor* in the Tehachapi Mountains (Fig. 1). The internal anatomy of the Caliente snail is unknown, but shell size and form is similar to *H. concolor*, though somewhat lighter in color with denser, more regular shell papillation. A small sample size precluded discriminant analysis of the shells.

Sonorelix (Fig. 7): This genus was described by Berry (1943) based on its lack of a dart sac and accompanying mucus glands, and an embryonic shell with anastomosing ridges. The superficially similar *Eremarionta* and *Cahuillus* usually possess dart sacs and mucus glands, and have embryonic shells ornamented with well-spaced, spirally arranged and elongated papillae. SEM imagery of the embryonic whorls of *Sonorelix baileyi* shows the reticulate (anastomosing) pattern of ridges characteristic of the genus and described as "sub-retiform" (ibid) (Fig. 7C). Based on morphology and geographical range, we consider the species sampled to be *Sonorelix baileyi* (Bartsch, 1904), the type locality of which lies on private land in Inyo County, about 8 km from our closest collection. Unfortunately, we did not have access to the type locality. The Sterling Hills site extends the known range of *S. baileyi* 6 km southward to a new station in San Bernardino County.

The reproductive tract of *Sonorelix* was described by Berry (1943) based on four taxa: (*S. borregoensis* Berry, 1929; *S. b. ora* Willett, 1929; *S. rixfordi*, Pilsbry, 1919; and *S. avawatzica* Berry, 1930). A major characteristic of these species is their lack of a dart sac and accompanying mucus glands. They exhibit a very long vagina with a unique muscular node, a long spermathecal duct with a robust diverticulum preceded by a very short common duct, a short epiphallus with a well-developed cecum, and a penis that is abruptly set off and enlarged from the epiphallus. All of these conditions are clearly shown in *S. baileyi*. However, Berry also mentioned and illustrated an excessively large "spermatotheca" (= spermatheca) and the penis containing a short conical verge. By comparison, *S. baileyi* has a moderate-sized spermatheca and an oblong, cylindrical verge (Fig. 7A and D).

Chamaearionta aquaealbae (Fig. 4): Our collections of this taxon are from the vicinity of Whitewater Canyon, for which this species is named. This canyon is located in the transition zone between the coastal slope and the northwestern edge of the Coachella Valley/Colorado Desert. The specimen sequenced was taken 6 km to the southwest of the type locality at the mouth of Cottonwood Canyon. We have extended its range by one canyon to the northeast of Whitewater,

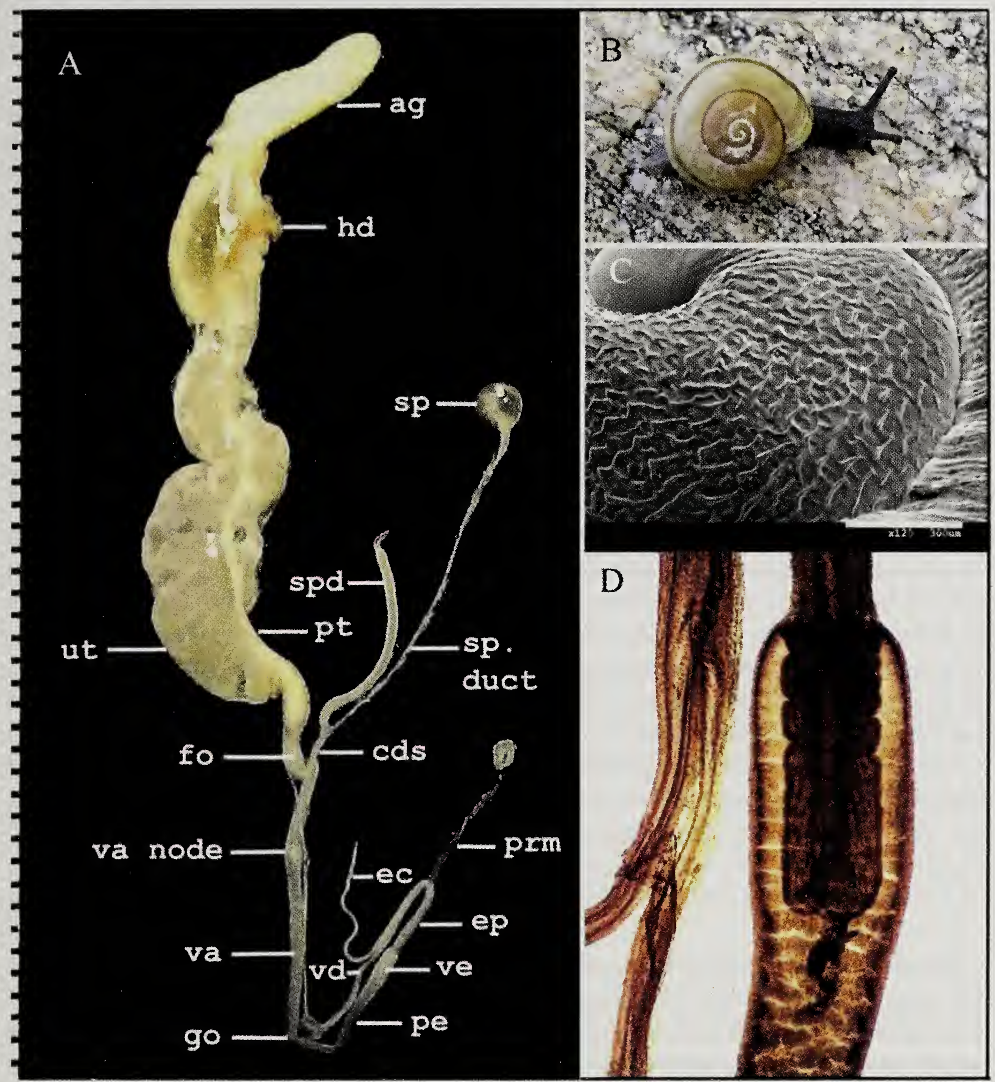

Fig. 7. *Sonorelix baileyi*. A. Freshly dissected entire reproductive tract (ex. ovotestis). Scale in mm. B. Live snail, shell diameter = 13 mm. C. SEM of embryonic whorls (in part), scale = 300um. D. Male anatomy (in part) showing verge (verge = 1.2 mm). Material leaving verge may be part of a spermatophore. Anatomical and SEM specimens, LACM 178951. Abbreviations: fo, free oviduct; ec, epiphallic cecum; others as in Table 6 or Fig. 6.

to that of Mission Creek about 4 km away. A shell from the Mt. San Jacinto foothills behind Cabezon is the only locality from the south side of San Gorgonio Pass. Whitewater Canyon is the furthest east and most desert-like station for the widespread coastal species *Helminthoglypta tudiculata* (A. Binney, 1843) as determined by museum records, and the furthest west station for *Eremarionta morongoana* (Berry, 1929) (this study). *C. aquaealbae* has been found under shrubs in leaf litter, piles of buried branches in gullies and under rocks in shaded locations.

Eremarionta/Cahuillus: The type species for *Eremarionta* appears to be *E. desertorum* (Pilsbry and Ferriss, 1908) from southwestern Arizona, though its taxonomic history is somewhat murky. The genus *Cahuillus* was recently erected from *Eremarionta* to account for a difference in the shape and comparative length of the double-walled portion of the epiphallis (Roth 1996). The two *Cahuillus* species accessed from GenBank, *C. unifasciatus and C. fultoni,* have the characteristic anatomy of that genus (Gilbertson et al. 2013). Our sample includes mtDNA sequences from several immature snails that could not be accurately determined on morphological grounds as *Cahuillus* or *Eremarionta*, and even in adults, the distinguishing characters are often difficult to see in dissected, slide-mounted reproductive tracts. Therefore, we refer generally to Groups 4-7 snails as *Eremarionta/Cahuillus.* The named species of *Eremarionta* in our study have not been proven anatomically as belonging to one genus or the other, but to avoid premature taxonomic redesignation, are referred to as *Eremarionta*, their given name, until such time as their true generic identity is determined. The inclusion of *Cahuillus* sequences in our genetic analysis thus appears to render the genus *Eremarionta* paraphyletic, but until all of our "*Eremarionta*" are proven to possess reproductive tracts typical of the genus (and until *E. desertorum* is sequenced), this paraphyly must remain hypothetical.

Geographically, *Eremarionta/Cahuillus* are found throughout the eastern Mojave and the Colorado Deserts, with most described species and subspecies in California. New stations for Group 6 snails are the Kingston Mountains and foothills of the Spring Mountains in Nevada (Fig. 1). Additional populations will likely be found in suitable habitat in other eastern Mojave Desert mountains. *Eremarionta morongoana* (Group 7) has proven to be more widespread than previously thought, occupying a series of canyons across a range of at least 80 km (Fig. 2). All *Eremarionta/Cahuillus* occur in rockslides with deep talus surrounded by desert scrub. Some also occur at palm oases where they find shelter and moist soil under beds of fallen palm fronds, especially where tumbled rocks are also present, such as BCC = Bear Creek Canyon in Group 5 and *Eremarionta millepalmarum* in Group 7). Shell morphology and color vary subtly between the various *Eremarionta/Cahuillus*, with the basic form of a smooth, flattened, tan shell with a thin brown shoulder band and a blackish body (see Discussion for comments on convergence and conservation of shell characters).

Discussion

The use of molecular markers in systematics is now standard (Avise 2004) and has led to the discovery of previously unknown biological diversity, including cryptic species (Pfenninger and Schwenk 2007, Jockusch et al. 1998, Hanken 1999). DNA barcoding for evidence of speciation became increasingly popular (Hebert et al. 2003), in part due to the need to quickly document biodiversity in the face of environmental degradation (Vietes et al. 2009, Fouquet et al. 2007). Hebert et al. (2003) proposed a 3% divergence in COI as a suggested "universal" species lower limit. However, 3% divergence is unlikely to be universal across all taxa (Rubinoff et al. 2006).

Terrestrial pulmonate snails have proven to be particularly troublesome, and deep intraspecific divergence in the COI of these species may not be uncommon. Davison (2002) summarized the surprisingly large range of mtDNA and allozyme variation in land snails from many regions and discussed possible causes. In Davison's study, intraspecific divergence levels typically ranged from 3% to 15%. Some, but not all of the snails discussed by Davison with divergence levels greater than 10% are widespread species with very large populations, such as *Cepaea nemoralis* (Thomaz et al. 1996) and *Helix aspersa* (Guiller et al. 2001). These taxa might be expected to show high genetic variation stemming from their large population size along with local and ancient isolation of demes that subsequently diverged, as hypothesized by Thomaz et al. (1996). Of course, if one accepts the biological species concept, these diverse populations may actually represent cryptic species. Without reciprocal crossing data, it is hard to discount this alternative hypothesis.

One reason for variation in the divergence level associated with species limits is an underlying inconsistency in mtDNA substitution rates between taxa (Avise et al. 1992, Kessler and Avise 1985, Nabholz et al. 2008). Hayashi and Chiba (2000) found that divergence between clades of *Euhadra peliomphala* (Bradybaenidae) ranged as high as 9.5%. When correlated with known geological events, this translated to a higher than expected mutation rate of 10% per million years, that could only be partially explained by its complex history of colonization and isolation over a changing geographical landscape. Their conclusion was that *E. peliomphala* mtDNA mutates considerably faster than expected. Weaver et al. (2006) adopted the rapid mutation rate of 6% per million years based on the works of Yamazaki et al. (1997) and Wethington and Guralnick (2004).

Not all studies of land snails have found evidence of high mtDNA mutation rates or mtDNA divergence. For instance, Hugall et al. (2002) found similar substitution rates between vertebrates and the tropical land snail *Gnarosophia bellendenkerensis,* and Ketmaier et al. (2010) found an average intraspecific divergence level of 3.6% + −0.2% in the land snail *Solatopupa guidoni*, a level comparable to that of vertebrates. Köhler and Johnson (2012) reported on mtDNA divergence (16S, COI) in Australian land snails in the genus *Amplirhagada.* They found that about 6% divergence was both the upper limit for clear morphological species as well as the lowest interspecific distance. They suggested that in their snails, the divergence range of 5–7% indicated "young" or incompletely differentiated species, which needed to be correlated with consistent morphological differences for evidence of full speciation. Davison et al. (2009) summarized much of the published COI data for land snails. They found no consistent "barcoding gap" or threshold between intra- and interspecific taxa. Their synthesis, (using Kimura-2 parameter distances) found a mean intraspecific distance of 2.5–2.6% and a mean interspecific distance of 10.0–11.8%, but there were many instances of high intraspecific divergence as well as low interspecific divergence. We used these percentages as broad indicators of genetic distances that might denote species level differences between helminthoglyptids, but much like Köhler and Johnson (2012) and Davison et al. (ibid.) recommended, we adhere to a combined approach using DNA along with morphology to inform taxonomic decisions, as exemplified by Kelly et al (2007) for chitons.

Species limits in Helminthoglypta. – Helminthoglypta is the only genus from the snails we sampled that has previously been sequenced. Roth, Lindberg and Cordero initiated the first in-depth work on *Helminthoglypta* (subgenus *Helminthoglypta*) that synthesized morphological and molecular data. Their studies of snails in northern California and southern Oregon (contained in unpublished reports: Roth 2002,[1] Lindberg and Cordero 2002[2]) uncovered cryptic species that are clearly differentiated by molecular markers (mtDNA COI, 16S) and have validated other species previously delineated only by shell and internal anatomy characters. Roth's 2002 report shows interspecific differences ranging from 12.7% to 20.0%. While intraspecific difference was less than 2% in 5 of 6 described or proposed species, the provisional Western Trinity clade contained two specimens that differed at 6.2% and 10.7% respectively from three other samples that differed less than 0.7% from each other. Lindberg and Cordero's 2002 report analyzed essentially the same data set, and found interspecific distances of 11%–23%, and typical intraspecific distances of 1.2% to 2%. Roth and Lindberg have recently begun additional analysis of their data, and Roth (pers. comm.) has indicated that as part of their new research, the variable Western Trinity clade will be analyzed anew with additional specimens. To summarize the data available at this time, most but not all analyzed *Helminthoglypta* species have an intraspecific range of 2% or less, and a lower interspecific threshold of 11–12%. "Troublesome" taxa fell in the 6.2–10.7% range.

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[1] Roth, B. 2002. Unpublished memorandum. Taxonomy and Classification of *Helminthoglypta*: BLM Purchase Order HAB020406. Corvallis Forestry Sciences Laboratory.

[2] Lindberg, D., and A. Cordero. 2002. Unpublished report. Molecular phylogeny of some land snails of the clades *Monadenia* and *Helminthoglypta* in Southern Oregon and Northern California. Report to USDI BLM, Roseburg, Oregon.

Most of the following discussion is limited to the terminal groups from our phylogenetic analyses, which deal with subgeneric classification levels. It would be premature to use this study to comment extensively on the higher level relationships between helminthoglyptid genera, because of weak support through the center of our analyses (Fig. 3). For example, the higher level relationships of *Sonorelix* and *Sonorella* have been studied and discussed in depth due to their similarity in reproductive structure, but there is no consensus on whether they are both simplified due to homoplasy or are closely related in lineage (Miller and Naranjo-Garcia 1991, Roth 1996). In our study, their status as sister taxa received some support in ML analyses (73%), but this relationship was lost in MP analyses. A better understanding of their phylogentic position relative to each other, and to the other helminthoglyptid genera, will require a robust genetic investigation beyond the scope of our current study.

In our analyses, the *Helminthoglypta* subgenus *Coyote* is not the sister group to *Helminthoglypta sensu stricto*, as represented by *H. umbilicata*, but rather to the group containing the remainder of the included helminthoglyptid genera. This could be an artifact of limited sampling of the *Helminthoglypta s. s.*, and/or our choice of a somewhat distant outgroup taxon, *Xerocrassa*. We felt that *Xerocrassa* was the closest taxon for which comparable sequence data was readily available (in GenBank), but acknowledge that a different outgroup may have resulted in a monophyletic *Helminthoglypta*. That said, discarding the *Xerocrassa* sequences and conducting unrooted analyses also did not result in support for a monophyletic *Helminthoglypta* (data not shown). Within our *Coyote* group (Group 1), most of the geographically isolated taxa have good bootstrap support, which corroborates the current taxonomy, recognizing *H. isabella*, *H. greggi*, *H. micrometalleoides* and *H. concolor* as valid species. By accepting *H. concolor* and *H. micrometalleoides* as distinct species based on their distinct differences in ecology and shell and internal morphology (see species accounts), we set the minimum COI interspecific difference for this subgenus at 7.8% (Table 1).

The recognition of the Jawbone Canyon population as a distinct species may be warranted, but it is relatively closely related to *Helminthoglypta isabella* (6.9% different in COI) and quite similar in appearance. Unlike *H. isabella,* the Jawbone Canyon snail is a true desert dweller, but lives at the extreme edge of the Mojave Desert only 3 or 4 km from slightly higher but still fairly arid slopes similar to those occupied by *H. isabella,* about 48 km further north. Despite the partial statistical differentiation of shell morphology between *H. isabella* and the Jawbone Canyon population (Fig. 5), the general unreliability of shell morphology in the taxonomy of pulmonates casts a shadow over its utility in this case. See (Goodfriend 1984) for a summary of shell variation, and (Köhler and Criscione 2014) for examples of widespread convergence and parallelism in shell form in western Australia camaenids). For now, the specific identity of the Jawbone snail remains undetermined, at least until the internal anatomy of *H. isabella* is described, and more intervening areas in the Piute Mountains are thoroughly searched.

Helminthoglypta "Caliente" is still mostly unknown. Although genetically and morphologically similar to *H. concolor*, additional study will be required to determine its specific status. It is not unexpected to find populations such as those in Caliente and Jawbone canyons that do not fit neatly into a category of species, and could represent "young" or incipient species.

Helminthoglypta micrometalleoides presents two interesting questions. First, the genetic clustering of *H. micrometalleoides* with *Helminthoglypta concolor* is unexpected, in that the geographically closer Jawbone *Helminthoglypta* is "skipped over" (Fig. 1), suggesting a complex geological history to the common ancestors of these snails. Secondly, even though *H. micrometalleoides* is consistently the smallest of the *Coyote* snails in its natural habitat, a shell series at SBMNH of *H. micrometalleoides* from Red Mountain shows variation in shell size between small wild individuals and individuals lab-reared by Walter Miller that grew as large as *H. greggi* (but nowhere near the size of *H. concolor).* Red Mountain and the El Paso Mountains are drier and hotter than those occupied by the larger foothill and montane *Coyote* species. Such an extreme environment could be expected to have selected for smaller snails that could either delve deeper into smaller crevices, mature sooner at a smaller size, or both. The desert *Coyote* species including *H. micrometalleoides*, *H. greggi* and several central Mojave species, are notably smaller than the montane and foothill *Coyote* species. Selection for small size could be counterbalanced by retaining a degree of phenotypic plasticity to respond to more favorable environmental conditions when they occur. See (Anderson et al. 2007) for an example of environmentally induced variation in shell size.

Group 7 snails, (*Eremarionta morongoana* and *E. millepalmarum*) despite being found in a number of adjacent canyons are found only in particular microrefugia, and gene flow between canyons is extremely unlikely under current climatic conditions. Given the variation in shell form and size exhibited by these snails from different canyons, it appears that *E. morongoana* is undergoing active differentiation. The furthest west populations of *Eremarionta morongoana* are quite similar to each other in form as well as in genetic uniformity: Whitewater, Painted Hills, Mission Creek, Dry Morongo (type locality), and Little Morongo all show typical *morongoana* form (and mtDNA uniformity), while populations further east vary from location to location. We are tentatively assigning all populations of Group 7 to *Eremarionta morongoana,* including *E. millepalmarum.* While this population is somewhat more isolated geographically from the other members of Group 7, it does not differ in mtDNA sequences to an extent justifying specific status when compared to divergence levels within Group 6 and between Groups 6 and 7. If *E. millepalmarum* were to be retained as a full species, other forms such as that from Shaver's Well (SW) would warrant specific status as well, due to similar levels of genetic differentiation and distinct shell characteristics. This may indeed be appropriate, but a taxonomic decision concerning *E. millepalmarum* and other distinct forms of *E. morongoana* must wait until more morphological and nuclear molecular data is gathered.

The Little San Bernardino Mountains and Joshua Tree National Park form a portion of the north-south transition zone between the Mojave and Colorado Deserts. The Mojave/Colorado desert ecotone has been documented as a zone demarcating lineage breaks in small mammals, lizards, snakes and a toad (summarized in Wood et al. 2013), tarantulas (Graham et al. 2015) as well as the more obvious differences in vegetation and climate (Axelrod 1977). This transition zone is also evident for land snails, separating *E. morongoana* from *Cahuillus unifasciatus* to the northwest and *Sonorelix rixfordi* to the immediate north. The areas to the east of Joshua Tree National Park (Eagle Mountains, Coxcomb Mountains and further east) need to be thoroughly sampled for *Eremarionta/Cahuillus* to determine the degree of isolation and divergence between *E. morongoana* and snails in the East Mojave (Group 6), as well as other species in eastern Riverside County currently under study (Eernisse, Gilbertson, and Goodward, unpublished data). Within Group 7, the most divergent sample is the easternmost snail, from the southern edge of Joshua Tree National Park (maximum 3.8% difference in COI). Interestingly, of the Group 7 populations, this Joshua Tree snail was also the most similar to those in Group 6 (6.0% difference). Once the mountains further east are sampled, it could be that the apparent intra-interspecific gap of 3.8% to 6.0% will disappear, replaced by clinal variation. A Mantel Test was performed on Group 7, and the p-value was significant at the 0.05 level ($p = 0.0461$, 10,000 permutations), suggesting isolation by distance is a significant factor. Concurrent to this, lineage sorting and/or limited gene flow seems to be leading to distinctive populations, such as the snails described as *E. millepalmarum.*

Eremarionta rowelli is clearly a polyphyletic taxon. Species that are (or were) under the name *rowelli* appear in groups 4, 6 and the central Mojave *Cahuillus*. In the latter group, *Eremarionta rowelli unifasciatus* was recently transferred to *Cahuillus* and elevated to species status on the basis of reproductive tract characters and genetic divergence (Gilbertson et al. 2013). As an example of the discovery of cryptic species through genetic sequencing, *E. rowelli bakerensis* plus TR (Travertine) snails (Group 4) diverge basally with strong bootstrap support from the remainder of *Cahuillus/ Eremarionta* samples. Further study (in progress, LHG) of this taxon is predicted to show that it should be removed from *rowelli* and elevated to specific status. *E. r. bakerensis*, known only from one location in the Mojave Desert clusters closely with Travertine snails from the Santa Rosa foothills, 250 km distant. This distance spans the Salton Rift, a portion of the extension zone between the Pacific and North American Plates developed in the late Miocene-Pliocene (McQuarrie and Wernicke 2005, Stock and Hodges 1989), the transition between the Colorado and Mojave Deserts, and the late Pleistocene Lake Mohave, present from 14-9 ka (Enzel et al. 2003).

Despite these isolating barriers and formidable distance, our samples of *Eremarionta rowelli bakerensis* and the Travertine Rock population are genetically very similar with regards to mtDNA markers (0.7% difference). If these two populations are remnants of a previously widespread species that has been sundered and reduced in range, we would still expect a higher degree of divergence similar to those exhibited by all the other sampled taxa. Without direct evidence, we are reluctant to invoke recent relocation by humans or other vertebrates as a possible explanation for the genetic similarity, but it is mentioned as a possibility since there is precedent for this process in Europe (Jesse et al. 2011, Grindon and Davison 2013).

Conclusions

Desert and foothill land snails in the family Helminthoglyptidae have proven to be more widespread than the literature and museum collections would indicate. Nearly all taxa are allopatric and occur in isolated microhabitat patches. All of the desert snails are found in the same basic climate regime and habitat type (talus and rock piles), and do not exhibit differentiating food preferences, at least in captivity. Mitochondrial DNA sequencing revealed hidden diversity with a wide range of genetic distances. Over comparable geographic distances, *Helminthoglypta* (*Coyote*) taxa tend to have greater genetic distances between them than *Eremarionta* and *Cahuillus*. The wide range of genetic distances with no consistent "barcoding gap" made it difficult to make taxonomic assignments. In the subgenus *Coyote*, named species based on morphology were corroborated with mtDNA analysis, but two newly discovered populations were ambiguous in both genetics and morphology. In the genera *Eremarionta* and *Cahuillus*, some taxa appear different in morphology yet are close in genetic distance, while others are genetically distinct but morphologically similar. It is unclear what species concept might be the most appropriate model for these helminthoglyptids. To test the biological species concept, reciprocal crossing trails would of necessity be conducted under artificial conditions, potentially bypassing behavioral reproductive barriers, and would be technically challenging since these snails often take four or more years to mature in captivity under natural seasonal activity patterns (pers. obs. DG). We recommend further study of *H. micrometalleoides*, including additional collections of the population at Red Mountain and rearing experiments to replicate those of Walter Miller to further explore plasticity in shell size.

Observations on courtship and mating would be useful in determining how selective these snails are during their extremely limited activity periods. Sequences of nuclear genes or microsatellites could help determine if introgression has taken place or if lineages have been consistently isolated, and help clarify potential instances of paraphyly uncovered in this study, as well as the relationships between genera that still remain unclear. Additional morphological work is needed as well to fully elucidate paraphyly, particularly between *Eremarionta* and *Cahuillus*.

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Appendix I. Material Examined and Collection Data

Specimens are listed by genetic group (see Fig. 3), then alphabetically by taxon within the group. The numbers listed in parentheses following the taxon names are the numbers of specimens examined or collected per lot. Within each taxon, museum specimens utilized for measurements are listed first, as indicated by museum accession numbers.

LACM = Los Angeles County Museum (Natural History Museum of Los Angeles County) SBMNH = Santa Barbara Museum of Natural History.

Newly collected specimens are listed next. These are in the collections of Goodward and Gilbertson and will be deposited at LACM and SBMNH. Collector is D. Goodward unless indicated otherwise. Specimens with names enclosed in quotes are of uncertain taxonomy. Museum vouchers of newly collected material are listed separately in Appendix II.

Group 1: *Helminthoglypta concolor* (1): holotype, SBMNH 34947, "Tejon Canyon, 10.3 road mi E of cemetery; in White Fir deadfalls", 03/07/1987. (2): paratypes, SBMNH 34950, 34948, same collection data as preceding. (2): under White Fir bark, El Paso Canyon, Tehachapi Mountains, Kern Co., CA. 34° 56' 52"N 118° 36' 26"W, 03/09/2015. (2): under White Fir bark, Cottonwood Canyon, Tehachapi Mountains, Kern Co., CA. 34° 57' 51"N 118° 36' 07"W, 03/09/2015. (2): GenBank accessions pending, "under White Fir bark and rock, vic. type locality, Tejon Canyon, Tehachapi Mountains, Kern Co., CA. 35° 0' 59"N 118° 30' 09"W, 03/09/2015. collector Mike White.

Helminthoglypta greggi (2): topotypes, SBMNH 128018, "Soledad Peak" 11/26/1944.

(2): topotypes, LACM G-2534, "3.5 miles south of Mohave, Kern Co. CA." 11/26/1944.

(1): SBMNH 11907, "hill 3 $\frac{1}{2}$ mi. S of Mojave; in rock slide" 11/29/1931. (2): SBMNH 142729, "N. slope of Soledad Mt.; under rocks" 11/26/1944. (2): SBMNH 11909,11908, "North slope Soledad Mt.; under rocks" 11/26/1944. (3): SBMNH 72926, "Soledad Mt, 3-4 mi S of Mojave, in rockslides along North slope" 11/16/1957. (6): LACM G-2535, "Under rocks, north slope of Soledad Mountain, Kern County California" 11/26/1944. (10): LACM G-7596, "Under rocks, north slope of Soledad Mountain, Kern County, Calif" 11/16/1957. (1): GenBank KY986341, north slope of Soldedad Mtn., Kern Co., CA. 34° 58' 50.6"N 118° 10' 41.5"W, 03/09/2012. (1): GenBank KY986340, museum voucher, see Appendix II.

Helminthoglypta isabella (3): SBMNH 72586, "along Highway 178, 3.8 miles NE of crossing with Kernville road at Isabella" 3/3/1957. (2): LACM G-2662, "under dead yucca, 2 mi. east of Isabella, Kern Co., CA, 9 June 1945." (1): LACM G-7497, "under dead yucca, south of highway 178, 3.8 miles east of New Isabella, Kern Co., CA, 3 November 1957." (4): 1.5 mi. south of Hwy.178, South Lake, Kern Co., CA. 35° 37' 14"N 118° 22' 06"W, 08/28/2015. (1): GenBank accession pending, Squirrel Valley, Mountain Mesa, Kern Co., CA. 35° 36' 34"N 118° 24' 12"W, 12/09/14.

Helminthoglypta "Jawbone" (3): GenBank KY986336, KY986337, KY986338, northern base of White Mountain, Jawbone Canyon, Kern Co., CA. 35° 17' 46"N 118° 08' 47"W, 4/12/12. (1): GenBank KY986339, Jawbone Canyon, Kern Co., CA. 35° 18' 49"N 118° 05' 04"W, 04/27/12. (1): Jawbone Canyon, Kern Co., CA. 35° 18' 49"N 118° 05' 04"W, 06/24/13. (2): Jawbone Canyon, Kern Co., CA. 35° 18' 59"N 118° 05' 02"W, 12/04/14. (1): Jawbone Canyon, Kern Co., CA. 35° 18' 07"N 118° 05' 30"W, 03/04/14. (2): Jawbone Canyon, Kern Co., CA. 35° 18" 49"N 118° 05' 06"W, 12/04/14. (6): Jawbone Canyon, Kern Co., CA. 35° 17" 21"N 118° 06' 46"W, 03/15/15.

Helminthoglypta "Caliente" (1): GenBank accession pending, Caliente Creek Rd. 6.3 mi. east of Bodfish turnoff, Kern Co., CA. 35° 18' 25"N 118° 29' 38"W, 12/3/14.

Helminthoglypta micrometalleoides (5): SBMNH 6885, "Red Mountain; in rockslides on N. side of a northern spur, near town off US 395." 12/16/1977. (11): type locality, Iron Canyon, El Paso Mountains, Kern Co., CA. 35° 26' 36"N 117° 47' 32"W, 10/24/2015.

(2): Specimen #1: GenBank KY986342, type locality, Iron Canyon, El Paso Mountains, Kern Co., CA. 35° 26' 36"N 117° 47' 32"W, 04/13/2013. Specimen #2: museum voucher, same collection data as preceding, listed in Appendix II.

Group 2: *Sonorelix baileyi* (2): Specimen #1: GenBank KY986344, museum voucher, listed in Appendix II. In talus, south side of Old Spanish Trail, Emigrant Pass, Nopah Mountains, Inyo Co., California, 35° 53' 10"N 116° 04' 13"W. 19 December 2012. Specimen #2: GenBank KY986345, same location and date as above. (2): GenBank KY986346, KY986347, Narrow canyon just west of abandoned limestone mine, S of West Talc Road, Sterling Hills, San Bernardino Co., CA.

35° 47' 20"N 116° 07' 45"W, 02/09/12.

Group 3: *Chamaearionta aquaealbae* (2): Specimen #1: GenBank KY986349, Under *Chilopsis linearis* trees, access road adjacent to Cottonwood Wash, 1.3 km NNE of Haughen-Lehmann Way Exit, I-10, Riverside Co., 33° 56' 08"N 116° 41' 12"W. 9 April 2013. Specimen #2: GenBank KY986348, museum voucher listed in Appendix II. Same location and date as preceding.

(2): Road to Mesa Wind Area, east of Cottonwood Canyon, near Pacific Crest Trail crossing, Riverside Co., CA. 33° 56' 57.5"N 116° 40' 59.2"W, 02/02/2013. (1): Specimen lost, Mission Creek Canyon approx. 6.7 km. above Mission Creek Preserve lower parking lot, San Bernardino Co., CA. 34° 02' 50"N 116° 39' 32"W, 04/03/2011.

(1): foothills of Mt. San Jacinto, 5.7 km. SE of Cabazon, Riverside Co., CA. 33° 53' 48.7"N 116° 43' 48.3"W, 02/07/2017.

Group 4: *Eremarionta rowelli bakerensis* (2): GenBank KY986350, Vicinity of type location, base of limestone hill 2 km NW of Hwy 127 exit/I15, Baker, San Bernardino Co., CA. 35° 16' 39"N 116° 05' 11"W, 02/26/2013.

Eremarionta "Travertine Pt." (2): GenBank KY986351, KY986352, base of Santa Rosa Mtns., 1.8 km W of Monterey Ave. exit, Hwy 86, Desert Shores, Imperial Co., CA. 33° 24' 08"N 116° 03' 52"W, 01/27/2013.

Group 5: *Eremarionta/Cahuillus* "Martinez Canyon" (2): GenBank KY986353, KY986354, Northern base of isolated rocky hill 1.1 km. W of the end of 72nd St., Thermal, Riverside Co., CA. 33° 31' 33"N 116° 11' 39"W, 11/21/2011.

Eremarionta/Cahuillus "Bear Creek Canyon" (2): GenBank KY986355, KY986356, 40 meters in from mouth of incised reach of Bear Creek Canyon, 2.5 air miles SW of Trailhead on Calle Tecate, La Quinta, Riverside Co., CA. 33° 37' 43.3"N 116° 19' 27.3"W, 11/12/2011.

Group 6: *Eremarionta* "Pahrump" (1): GenBank KY986357, Spring Mtn. foothills, 560 m N of Carpenter Canyon Rd., Nye Co., NV. 36° 10' 19"N 115° 50' 00"W, 04/02/2013.

Eremarionta rowelli acus (1): GenBank KY986358, 10 miles S of Needles, side canyon E side of Hwy. 95, San Bernardino Co., CA. 34° 40' 44"N 114° 37' 16"W, 12/14/2011.

Eremarionta "Kingston" (1): GenBank KY986359, 200 meters E of Smith Spring, Kingston Mtns., San Bernardino Co., CA. 35° 47' 15"N 115° 59" 44"W, 11/29/2012. (1): GenBank KY986360, edge of Omega Mine, 2 km. ESE of Excelsior Mine Rd., Kingston Mtns., San Bernardino Co., CA. 35° 47' 12"N 115° 58' 19"W, 12/13/2012.

Group 7: *Eremarionta millepalmarum* (2): GenBank KY986361, KY986362, under rocks and palm fronds, Biskra Palms Oasis, Indio Hills, Riverside Co., CA. 33° 47' 23"N 116° 14' 58"W, 03/01/2012.

Eremarionta morongoana (1): GenBank KY986363, East Wide Canyon, side canyon 400 m NE of end of Hilltop Rd., 2.3 km. N of Dillon Road, Riverside Co., CA. 33° 55' 56"N 116° 22' 34"W, 01/23/2012. (1): GenBank accession pending, base of slope, 280 m. W of Cottonwood Springs Rd., Joshua Tree National Park, Riverside Co., CA. 33° 43' 06.7"N 115° 48' 47.1"W, 02/11/2015.

(2): GenBank KY986364, KY986365, small rockslide south side of Box Canyon Rd., across road from Shavers Well, Mecca Hills, Riverside Co., CA. 33° 37' 06"N 115° 55' 02"W, 04/16/2012.

(2): GenBank KY986366, KY986367, Base of hill, east branch of Yellow Spots Canyon, 1.7 km. N (by air) of Dillon Rd., Riverside Co., CA. 33° 43' 26.5"N 116° 01' 49"W, 04/02/2012.

(3): Specimen #1: GenBank KY986368, steep NW-facing slope, Fargo Canyon Rd., 2.9 km NE from Aqueduct Rd., Riverside Co., CA. 33° 45' 47"N 116° 04' 60"W, 03/03/2012. Specimen #2: GenBank KY986369, juvenile hatched from egg laid by adult collected at preceding location and date. Specimen #3: same collection data as preceding. (1): GenBank KY986370, under rock beneath shrubs, small embayment on east side of Whitewater Canyon, 900 m SE of Whitewater Canyon Preserve entrance, Riverside Co., CA. 33° 58' 58"N 116° 38' 58"W, 11/16/2011. (1): GenBank KY986371, base of conglomerate outcrop, west side of Super Canyon, Painted Hills, Riverside Co., CA. 33° 56' 54"N 116° 37' 31"W, 01/23/2012. (2): GenBank KY986372, KY986373, southern bank of Mission Creek Wash, 250 m SSE of Mission Creek Preserve gate, Riverside Co., CA. 33° 59' 55"W 116° 36' 44"W, 12/28/2011. (1): GenBank KY986374, south side of mouth of Little Morongo Canyon, 1.7 km. (by air) NE of intersection of Mission Lakes Blvd. and Little Morongo Rd., Riverside Co., CA. 33° 59' 25.5"N 116° 31' 11"W, 01/09/2012.

Appendix II. Museum Voucher Specimens

1. *Sonorelix baileyi* — LACM 178951. GenBank KY986344, shell, SEM shell, preserved (EtOH) soft anatomy (minus reproductive system), and slide-mounted reproductive system (all but the SEM shell are the same individual). In talus, south side of Old Spanish Trail, Emigrant Pass, Nopah Mountains, Inyo Co., California, 35° 53' 10"N 116° 04' 13"W. Coll. D. Goodward, 19 December 2012. Slide: L. Gilbertson.
2. *Helminthoglypta (Coyote) greggi* — LACM 178952. GenBank KY986340, SEM shell and slide-mounted reproductive system. Southernmost outcrop of Soledad Mtn., 0.75 km. N. of Backus Rd., 0.41 km. E of 40th St., Kern Co., 34° 57' 27.4"N 118° 11' 55.8"W. Coll: D. Goodward, 3 September 2012. Slide: L. Gilbertson.
3. *Helminthoglypta (C.) micrometalleoides* — LACM 178953. GenBank KY986343, slide-mounted reproductive system. Topotype, "S side Iron Canyon Rd., 3 mi. up canyon from junction with Garlock-Goler

highway", El Paso Mtns., Kern Co., CA. 35° 26' 36"N 117° 47' 32"W. Coll: D. Goodward, 13 April 2013. Slide: L. Gilbertson.

4. *Chamaearionta aquaealbae* — LACM 178954. GenBank KY986348, SEM shell.

   Under *Chilopsis linearis* trees, access road adjacent to Cottonwood Wash, 1.3 km NNE of Haughen-Lehmann Way Exit, I-10, Riverside Co., 33° 56' 08"N 116° 41' 12"W. Coll: D. Goodward, 9 April 2013.

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Endlicher and Sequoia: Determination of the Etymological Origin of the Taxon *Sequoia*

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The genus *Sequoia* owes its taxonomic identity to Austrian botanist Stephen L. Endlicher (Fig. 1). Research of primary material in Vienna and other locations have revealed Endlicher as a gifted linguist and botanist, who corresponded and interacted with colleagues throughout the world. These included persons who were experts on both the Cherokee language and the person Sequoyah. Endlicher's botanical work of creating eponymous taxa combined with his knowledge of the person Sequoyah throws new light on the origin of the genus *Sequoia*.

The coast redwood (*Sequoia sempervirens*) and the giant sequoia (*Sequoiadendron giganteum*) share more than their immensity and co-occupation of California. The root genus, *Sequoia*, has presented an intriguing taxonomic origin question since the moment it was assigned by Stephan Ladislaus Endlicher in 1847 (St. John and Krauss 1954). Assumptions have been made that Endlicher assigned the name to honor the Cherokee linguist, Sequoyah, who had died just five years before. In 2012, Gary Lowe made an intuitive case for *Sequoia* being from the Latin "sequor" (to follow). I traveled to Austria in an effort to resolve this puzzle through an exhaustive review of primary sources, including original works in libraries and museums; a review of Endlicher's publications, correspondences, journals; and notes of persons who knew and interacted with him. My findings suggest that Endlicher, a botanist, linguist, and communicator with other scientists interested in indigenous people of the Americas, used his expertise and pattern of naming plants after people to name the coast redwood after the man, Sequoyah.

Stephan Ladislaus Endlicher-Stephan Ladislaus Endlicher (1804–1849) was born in Pressburg, a German-speaking town in the Austro-Hungarian Empire in 1804. He studied theology and languages, and became a librarian. In 1828, Endlicher was appointed as a librarian to the National Library in Vienna and was placed in charge of the Handwriting (Handschriftin) Department. In addition to obtaining specimens for the collection he began his studies in medicine. At that time, medicine was not just the study of pathology, but of botany and pharmacology. Plants were the basis for cures and physicians consulted their *Materia Medica*, a primarily plant-based tome for patient treatment (Reidl-Dorn 2013). In addition to handwriting Endlicher developed an interest in maps, in Hungary, and in China. He became an expert in Sinology and furthered his remarkable linguistic ability. Over time he became proficient in Hungarian, Czech, German, French, Latin, Chinese, Italian, English, ancient language forms (he transcribed old German to new), and American Indian languages. After he joined the National Library, Endlicher pursued prime appointments in his chosen areas of expertise. In 18th Century Vienna, a person had to be a Free Mason to receive political appointments but by the middle of the 19th Century, family connections had become more important. Endlicher's wife Caecilie had a sister who was married to the Secretary State Chancellor, and this link to royal patronage opened many doors for Endlicher (Reidl-Dorn 2013; Stangl 2013).

Through his connections, Endlicher became personal tutor to Emperor Ferdinand, developing both a personal friendship with the Emperor and a good relationship with the members of the larger royal court. There he solicited letters of recommendation from colleagues to the crown

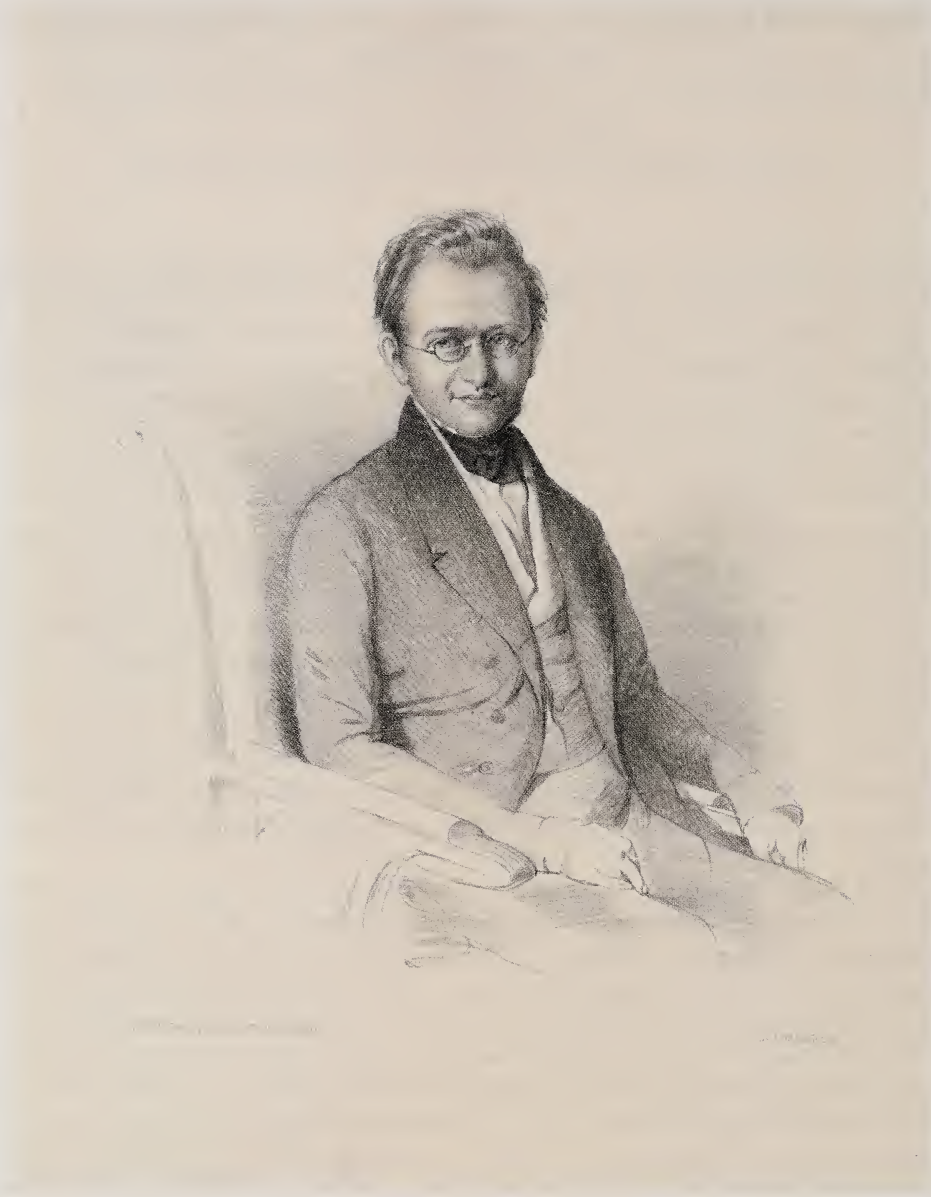


Fig. 1. Stephan Ladislaus Endlicher, early 1800's, Vienna Austria. (Courtesy of the National Library of Vienna).

for specific appointments. He soon received an appointment as the Director of the Botanical Gardens for the University of Vienna. He had the botanical collection of the National Museum sent to the Renweg Herbarium at the Botanical Gardens where he lived in a house on the grounds. His assistant for part of the time was Edward Fenzl who later succeeded Endlicher as Director of the same Botanical Gardens (Stangl 2013).

Botanical Eponyms-Among Endlicher's many pursuits were identification and classification of plants brought back from expeditions from around the world. One such set was from the Norfolk Islands. Working with this collection, Endlicher showed his propensity to name plant taxa for particular people. In *Prodromus Florian Norfolk* (1833) he named plants for the collector and Austrian botanical illustrator Ferdinand Bauer (1760–1826), (*Zehneria baueriana*). Another named plant for a person was *Bryonia affinis,* but the personal etymology is unknown (Endlicher 1833). Other examples of taxa named for persons by Endlicher include *Verticordia huegelii,* named by Endlicher in 1839 for Carl von Huegl (1795–1870), an Austrian Naturalist (George 2002); *Ungeria floribunda* in 1836 for Franz Unger (1800–1871), an Austrian Botanist (Schott and Endlicher 1832); and *Stirlingia* in 1837, for Sir James Stirling (1791–1854), first Lieutenant Governor of Western Australia (Quattrocchi 1999).

Asa Gray (1810–1888), the American naturalist wrote of Endlicher's naming a genus for a recently deceased person. Gray recalled in his journal of his visit to Europe, a conversation with Endlicher on why a plant was named:

> *Ungnadia* (the character of which Endlicher has not yet published, — the last plate in the "Atakta") was named in memory of Baron Ungnade, once an ambassador from Austria to Constantinople or Persia, I forget which, and the first to introduce *Esculus hippocastanum* into Europe, — hence the propriety of the name (Gray 1894).

Other than Gray's recollection of the dialog, there is no written record of this taxon's etymology by Endlicher. It was not common at this time for any taxonomist to record the reasoning behind the assignment of taxa. Lisa De Cesare, curator of the Harvard University archival collection of Gray's papers, notes that: "Gray wasn't too interested in preserving his correspondence during the early years of his career. It wasn't until he (Gray) married Jane and she took his correspondence in hand that [we find] the rich collection of letters appear" (De Cesare 2016).

Endlicher was unceasing in his work. He named or co-named over 1600 plants from the tropics alone (Tropicos 2016). In addition to assigning taxa designations for people, Endlicher also named taxa for specific characteristics of the plant itself. Endlicher worked with specimens that were sent to him as well as those already in the Botanical Garden's collection. Thaddeus Haenke (1761–1817), originally from an area now known as the Czech Republic, studied botany, medicine and minerology at the University of Vienna in 1780. He was a member of the Malaspina Expedition to the Americas in 1789. His work with the "Indianers" was extensive, learning the pharmacopeia of the plants and the indigenous people's uses of them. His collection was in Endlicher's hands (Bleichmar 2012).

Endlicher wrote works with Karl Martius (1794–1868) and Eduard Poeppig (1798–1868) on plants of Brazil and Chile (Martius, et al. 1840–1845). Eduard Poeppig, an Austrian botanist and naturalist explored Pennsylvania, Cuba, and South America and collaborated with Endlicher on two volumes of plant descriptions. Poeppig also studied North and South American Indian tribes and collected skeletons of indigenous people (Martin 1970; Poeppig 1839). Poeppig wrote to Endlicher about Indians and about the plants and people of North and South America (Reidl-Dorn 2013).

As a polyglot, Endlicher spoke with many scientists, and corresponded with many more. His fluency allowed him to pick a variety of words to indicate a noun or descriptor in German, Latin, English, French, Czech, Hungarian, Italian, and Chinese. He was one of the era's noted Sinologists and frequently corresponded with Peter Steven Du Ponceau (1760–1844) about the language, culture and history of China (Du Ponceau was a French linguist who served in the Continental Army during the American Revolution, was a Sinologist, and an expert on American Indigenous peoples' languages) (Du Ponceau 2016). Du Ponceau's authoritative knowledge of the indigenous languages of North America included the Cherokee syllabary created by Sequoyah (c.1776–1843, Fig. 3), the illiterate son of a Virginia Fur Trader father and Cherokee mother (Du Ponceau 2016; King 2016).

The Sequoia Connection-Sequoyah created a syllabary-based language for the Cherokee Nation, the only person known ever to perform such a feat (King 2016; Rhodarmer 2016). Sequoyah presented his syllabary to the Cherokee nation in 1821. The name "Sequoyah" has had a variety of spellings, an interpretation of the Cherokee, ᏎᏉᏯ ending in "ie," making it Sequoie or Sequoia (Rhodarmer 2016), a direct link to the extant genus name. Samuel Knapp (1783–1838), who personally interviewed Sequoyah spelled his name See-quah-ya (Knapp 1828). Sequoyah's English given name of George Gist also had a variety of spellings (Guess, Guest) (Rhodarmer 2016; Knapp 1828).

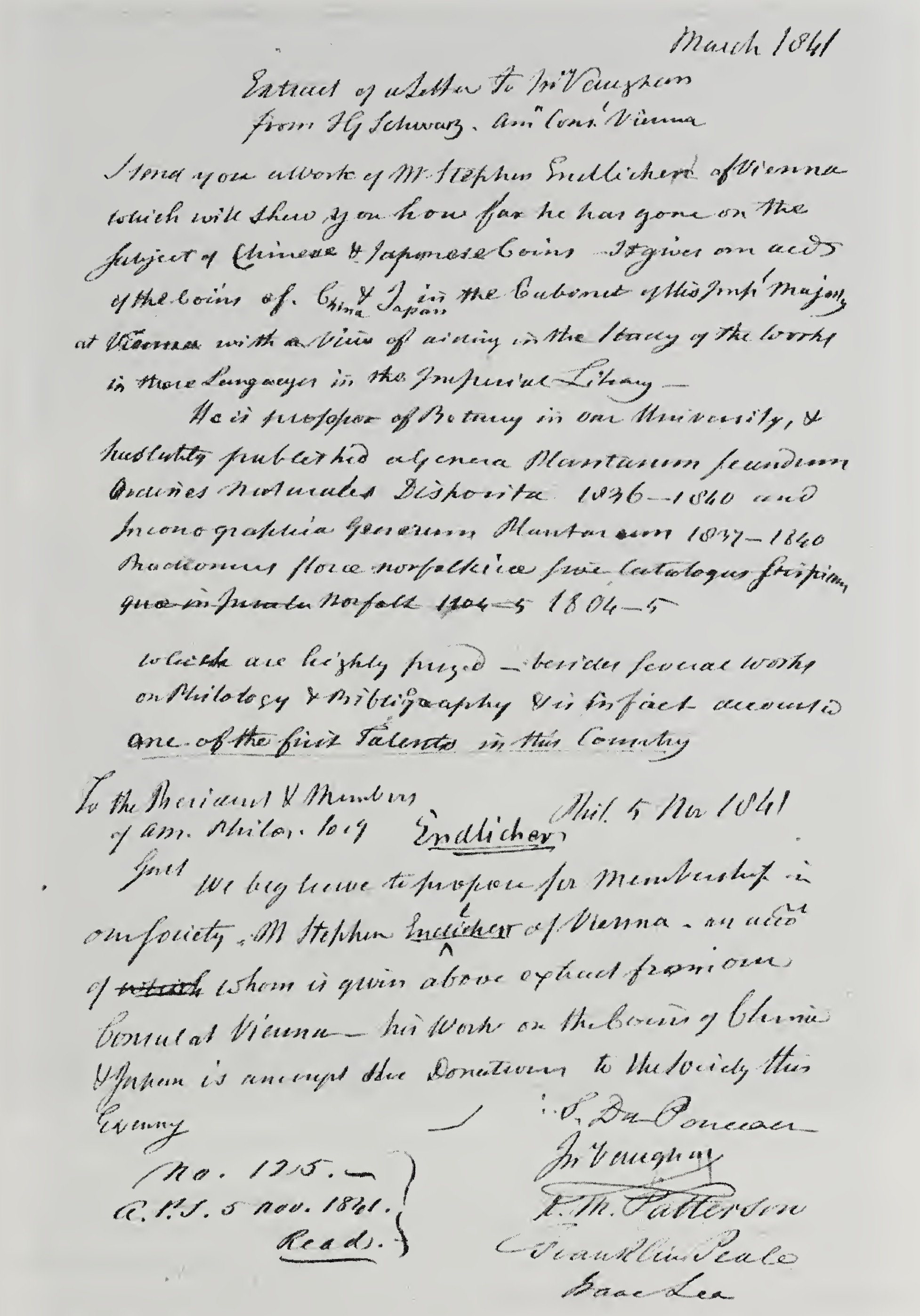
Endlicher's collaborator Du Ponceau's interest in Sequoyah was preceded by an interest he developed in indigenous cultures in the late 18th Century. Du Ponceau shared this interest through correspondence with Albert Gallatin (1761–1849), an ethnographer, linguist, and President Thomas Jefferson's Secretary of the Treasury. From 1801 to 1843, Du Ponceau and Gallatin discussed American Indian languages and linguistics and collaborated on a volume about Indian languages commissioned by Jefferson (DuPonceau 2016). Gallatin wrote *A Synopsis of North American Indian* Tribes in 1836. Therein he described the syllabary created by Sequoyah in great detail, including an analysis of the construction of the language itself (Gallatin 1836).

Gallatin, along with Thomas McKenney (1785–1859), American Superintendent of Indian Affairs in the mid-1820s, championed the cause of America's indigenous peoples, citing Sequoyah's work as an example of their intellectual abilities:

> Responding both to Congress's impending consideration of a removal bill and to a literary debate over the character of Native languages, the retired statesman Albert Gallatin convinced the executive branch to fund the collection and publication of linguistic materials in 1826. To Gallatin, 'all that belongs to human knowledge and its progress, to the formation of language 8c to political institutions is connected together and belongs to us.' Just weeks later, the director of the Indian office, Thomas L. McKenney, sounded a similar note. Since receiving news, about a year earlier, that the previously unlettered Sequoyah had invented a syllabic alphabet for the Cherokee language, McKenney had been concerned about its consequences for Indian progress, for he 'esteemed language to be the very centre of power that will reform and bless our Indians' (Harvey 2010).

Gallatin and Du Ponceau were noted collaborators promoting the need for understanding the implications of the alphabet invented by Sequoyah. Du Ponceau's correspondences with Endlicher covered more than just Sinology. The American Philosophical Society (APS), founded by Benjamin Franklin in 1743, and still in existence today, was and is an organization where one must be nominated for membership. Endlicher became member #1166, in March of 1841. Endlicher's nomination (Fig. 2), put forward by J.G. Schwarz, American Consul in Vienna, was confirmed by APS members Peter S. Du Ponceau, John Vaughan, R. M. Patterson, Franklin Peale, and Isaac Lea (Spamer 2016). Two of these, Du Ponceau and Vaughan, were authorities on the Cherokee language and great admirers of Sequoyah (Goodman and Swiggers 1994).

Another APS member and frequent correspondent with Endlicher was Asa Gray. On Gray's first visit to Europe in 1839, he visited Endlicher, staying in Vienna for 12 days. He described Stephan as "…extremely good-looking, and younger even in appearance than I expected, although Bentham told me he was about his own age; he looks about thirty-three. I had the



March 1841

Extract of a Letter to Mr Vaughan
from J G Schwarz. Amn Consl Vienna

I send you a Work of Mr Stephen Endlicher of Vienna
which will shew you how far he has gone on the
subject of Chinese & Japanese Coins It gives an acct
of the Coins of. China & Japan in the Cabinet of His Impl Majesty
at Vienna with a View of aiding in the Study of the works
in those Languages in the Imperial Library —
He is professor of Botany in our University, &
has lately published a Genera Plantarum secundum
Ordines Naturales Disposita 1836—1840 and
Iconographia Generum Plantarum 1837—1840
Prodromus florae norfolkicae sive Catalogus stirpium
~~quae in Insula Norfolk 1804—5~~ 1804—5

which are highly prized — besides several works
on Philology & Bibliography & is in fact accounted
one of the first Talents in this Country

To the President & Members
of Am. Philos. Socy   Endlicher   Phil. 5 Nov 1841
Gent We beg leave to propose for Membership in
our Society, Mr Stephen Endlicher of Vienna - an acct
of ~~which~~ whom is given above extract from our
Consul at Vienna — his Work on the Coins of China
& Japan is amongst the Donations to the Society this
Evening

P. S. Du Ponceau
Jn Vaughan
R. M. Patterson
Franklin Peale
Isaac Lea

(No. 125. —
A. P. S. 5 nov. 1841.
Read.)

Fig. 2. Endlicher's nomination to the American Philosophical Society and below, the signatories Du Ponceau, Vaughan, Patterson, Peale and Lea. (Courtesy of the American Philosophical Society).

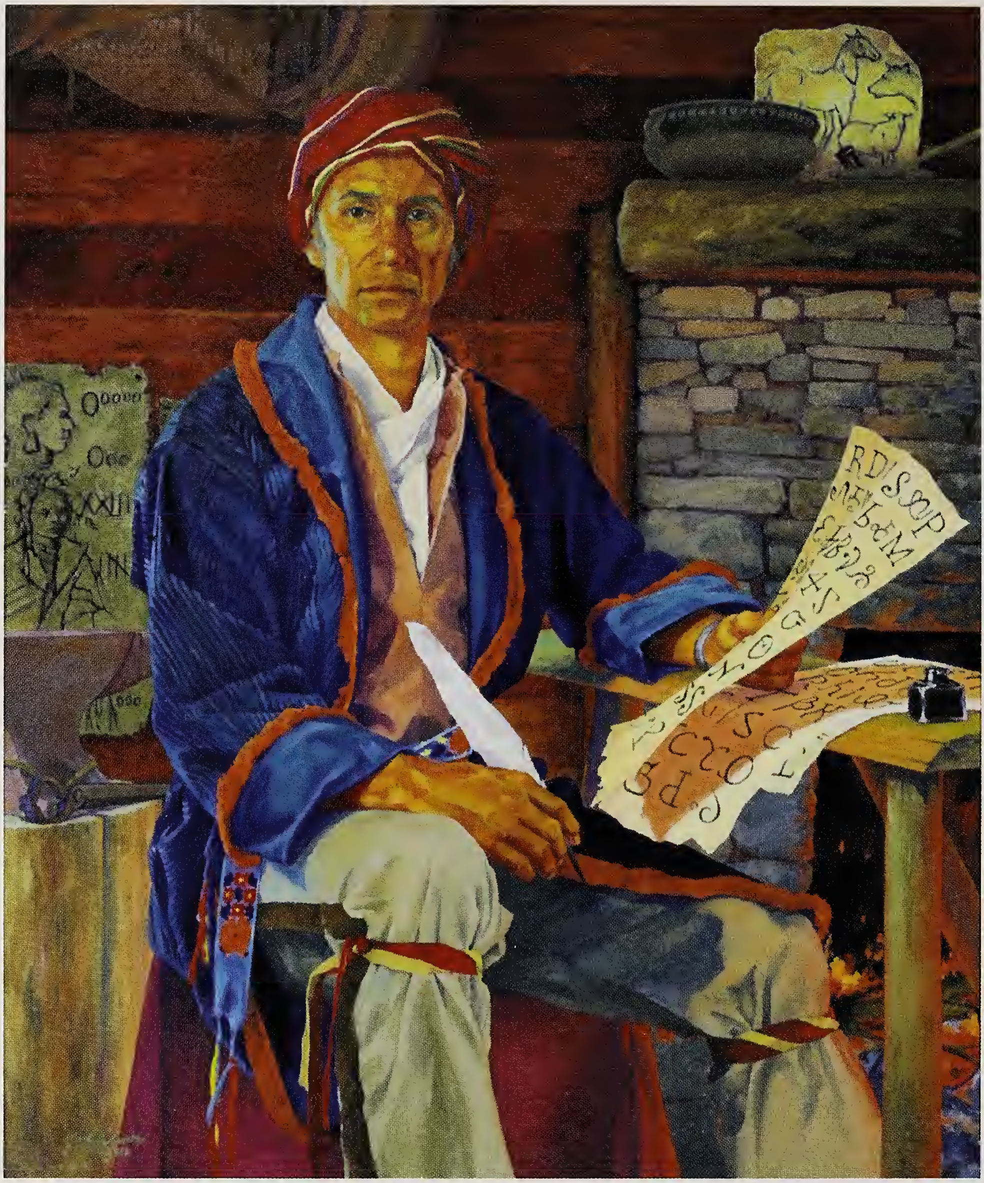


Fig. 3. Sequoyah. Portrait by Carlyle Urello (Courtesy of the Tennessee State Museum).

pleasure to present in person the copy of the *Flora* designed for him" (Gray and Torrey 1838–1843; Gray 1894). Gray's ability to reproduce his conversations with Endlicher and to, in essence, record Endlicher's thoughts is shown in a passage Gray wrote commenting on the strictness of publication in Austria at the time of his visit:

> Nothing can be printed and published here, without first being examined and approved by a censor of the press. The government appoints four or five persons in Vienna, who examine in different departments. . .. Every author must send his manuscript to the police-office, whence it is handed over to the proper censor, who certifies that it contains nothing

> immoral, nothing against the government, and that it is good literature, or science, or poetry, as the case may be, and worthy of being published; it is then returned to the author, with permission to print it... To my great surprise, Endlicher, who gave me all this information, informed me that all the manuscript of his '*Genera Plantarum*' is sent to the police, who transmit it to Baron Jacquin, the censor for natural history, etc., and who is well paid for the business, but who knows just as much about it as if it were written in Arabic...Endlicher spoke of all this in terms which there is no necessity for me to record just at present. He gave me an anecdote respecting the publication of his earliest botanical work of any consequence, a Flora of his native town, the "*Flora Posoniensis*" the manuscript being duly sent to Jacquin, that worthy refused to give it his imprimatur, because it was arranged according to the natural system! which Jacquin did not like; and Endlicher was obliged to apply personally to the ministers and take great pains, when he obtained permission to print in spite of the censor; he took his revenge by dedicating the work to Baron Jacquin himself! This system sufficiently explains the low state of literature in Austria, as compared with northern Germany. I could hardly believe all I have heard, had I not obtained my information from such authentic sources (Gray 1894).

By the 1840's Endlicher was working on a wholesale update of groups of plants. His *Generum Plantarum* was a turning point for him as a botanical scholar. His books were written in Latin and here he used the Latin "sequentia" to indicate "follow" ("Signa sequentia literis subposita sic intelligenda.") (Endlicher 1836–1840). In notes written in German, he emphasized the need to understand botany in order to understand pharmacopeia for medicinal uses. He also knew that understanding plant use by "Indianer" [Indians] was important, and specifically mentions North American plants and Indians: "wie in den Wäldern von Nordamerika Plantago major den europäischen Ansiedler verräth, daher diese Pflanze von dem eingeborenen Indianer (aboriginal indian)» die Fußstapfe der Weissen « genannt wird. Die rasche Ver" (Endlicher and Unger 1843).

His greater effort was with the *Synopsis Coniferum* (Endlicher 1847). Here he reviewed several genera and reclassified several, including *Taxodium sempervirens*, the extant genus of the Coast Redwood of California. There is no doubt that Endlicher changed the genus of *Taxodium* to *Sequoia*, but why *Sequoia*? Endlicher was familiar with other researchers' findings and taxonomies before undertaking a revision of the conifers in his *Synopsis*. This included the British Publication, *Description of the Genus Pinus* by Aylmer Bourke Lambert (1761–1842) with David Don (1799-1841) and an account of the Lambertian Herbarium by Lambert in 1824 (Don worked for Lambert as a botanist). Here Lambert with Don described the Coast Redwood as *Taxodium sempervirens*. First published in 1803 by Lambert, in the 1828 edition preface, Lambert explains that Don updated this edition with a description of *Taxodium sempervirens* (Lambert and Don 1824). One of the indicators of Endlicher's awareness of other researchers' works and his communication with their authors was his election as a "Foreign Member" of the Linnean Society of London on May 7, 1839. The American botanist John Torrey, was also inducted as a "Foreign Member" on this date. In the bound issue of the *Proceedings of the Linnean Society* (LSL 1839) were articles written by the famous English botanist George Bentham (1800–1884) and David Don.

Communication between scientists from around the world was commonplace and vital at the time. Fraser and Sellers (2014) note:

> By the middle of the 19th century, there was sufficient worldwide knowledge of plants for the development of a more elaborate plant classification system based on the differing features of the whole plant, enabling them to be grouped into families with common elements. By

> this time George Bentham. . .and Joseph D. Hooker. . . in England, Stephen Endlicher. . .in Austria, John Torrey. . . and Asa Gray. . .in America had with their worldwide contacts, developed plant classifications based on 'natural systems.'

In 2012, Gary Lowe, writing in *Fremontia: The Journal of the California Native Plant Society*, made a case for *Sequoia* being from the Latin "sequor" (to follow) for the species' place among the Cypress conifers. However, this analysis is in question. Mark T. Riley, a Professor of Classics Studies and Latin at the California State University, Sacramento, comments:

> The idea that this is the Latin word for sequence is false. It does look like it should be derived from the verb (and only a verb) sequor 'I follow.' Sequens means 'following' secutus means 'having followed' and so on. You can say 'in sequence' or 'sequentially' by 'per ordinem' (Riley 2016).

If Endlicher were to name the Coast Redwood for its place in a sequence, it would more properly have become *Sequentia sempervirens.*

Dr. Christa Reidl-Dorn, Department Head at the Natural History Museum of Vienna has studied Endlicher at the museum archives. She, and others in Vienna, e.g. Robert Stangl (2013), University of Vienna Director of the Botanical Library and Maria Petz-Grabenbauer (2013), an Endlicher scholar, Culture and Science Historian and professor, feel strongly that rather than simply applying a Latin term, Endlicher deliberately named *Sequoia* for the person. Dr. Reidl-Dorn noted in Endlicher's work with plants that he wrote on pharmacopeia and referred to "Seneca the Indianers" (Endlicher 1842). Reidl-Dorn (2013) argues that Endlicher would have been aware of the linguist Sequoyah.

Following an intense and thorough review of all primary papers related to Endlicher at his place of work and residence and in the archives in Vienna, Austria as well as works of those who wrote of him and corresponded with him allowed insight into the man. This knowledge revealed how his work was often centered on patronage, money, and notoriety. In particular, his motives for the assignment of *Sequoia* as a genus was revealed through his associations, communications with others and how they in turn often revealed his thoughts when he himself left no written details.

As fluent as he was in so many languages, his use of "sequor" solely as a word for 'to follow' would be an egregious error for such a scholar. Endlicher had already shown the proper use of the word for 'in a sequence' (sequential) and, in this matter, *Sequenta sempervirens,* would have been more correct and proper.

Endlicher knew of the person Sequoyah. There were stories in the German language newspaper about Sequoyah dated to his time period as well as English print stories. John William Parker's *Saturday Magazine,* Vol 20, April 23, 1842, had an extensive story on the person Sequoyah titled, "Ingenuity of a Cherokee Indian," that was in the Heidelberg Germany Library Archives. In addition, Endlicher corresponded with the very persons who highlighted the achievements of Sequoyah, his fellow members of the American Philosophical Society, Du Ponceau and Vaughan.

While there is nothing in any written work by or to Endlicher that states he named *Sequoia* either for its place in a sequence or for the person Sequoyah, we have seen he has discussed his motives for assigning taxa with colleagues who, in turn recorded them, e.g. Gray and *Ungnadia.* Gray was also responsible for recording Endlicher's motives for assigning the taxon *Sequoia.* Gray wrote a history of *Sequoia* and presented this as a talk in Dubuque, Iowa in 1872. He also edited a book of George Engelmann's work where Engelmann and Gray reiterated the origin of the genus *Sequoia* for the man, Sequoyah:

> SEQUOYAH
>
> In last Sunday's issue you revive the almost forgotten, though most interesting history of the invention of the Cherokee alphabet and written language by the half-breed, Sequoyah, and mourn that to-day no man can point out the spot where moulders the dust of the Cherokee Cadmus.
>
> His resting-place may be unknown, but his name and his memory live in the most magnificent vegetables of this continent. The mammoth tree of California has been claimed by English as well as Americans for their greatest men, and has been named by the former Wellingtonia and by the latter Washingtonia, but a celebrated Vienna professor, Endlicher, as eminent a botanist as he was a linguist, had already, in 1847, established a genus which comprises the mammoth trees as well as the scarcely less magnificent Red Woods of California, and had named it Sequoia, in commemoration of the aboriginal linguist; and as long as botanical science exists both these wonders of the western world will perpetuate the name of the Cherokee Cadmus. — Missouri Republican, Sept. 28, 1873. (Trelease and Gray 1887).

Endlicher's linguistic skills and knowledge allowed him to become a polyglot and student of world-wide languages. He corresponded with and knew people who studied the syllabary of Sequoyah. Endlicher also named many plant taxa for both scientists and persons of note. Endlicher knew and admired the work of the man Sequoyah. Once he realized the previously assigned *Taxodium* required a change in genus, this evidence supports that he honored the recently deceased Sequoyah, by assigning the genus *Sequoia* to the Coast Redwood.

Acknowledgements

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Late-season Reproduction in Western Toads (*Bufo boreas*)

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[2] *Santa Monica Mountains National Recreation Area, 401 W. Hillcrest Drive, Thousand Oaks, CA 91360*

Western toads (*Bufo boreas,* or *Anaxyrus boreas* of some authors) typically breed from late January to July depending on elevation, latitude, and local conditions (Sornborger 1979; Stebbins 2003; Thompson 2004; Muths and Nanjappa 2005). Generally, breeding takes place early in the active season and is explosive with the breeding season lasting only a few weeks with most of the breeding activity occurring in a few nights (Sornborger 1979; Olson et al. 1986; Muths and Nanjappa 2005; Pauly pers. obs.). At low elevation sites in Southern California, for example, breeding may begin as early as late January assuming rainfall has been adequate to fill breeding sites and stimulate activity. At higher elevation sites, breeding activity is triggered by warming conditions and snowmelt with toads breeding shortly after emerging from hibernation sites (Sornborger 1979; Olson et al. 1986; Fetkavich and Livo 1998; Hammerson 1999; Thompson 2004; Muths and Nanjappa 2005).

Here we report unusually late breeding activity in western toads. On 9 November 2015, one of us (KSD) observed late stage tadpoles (up to Gosner Stage 43) at a seasonal pond in the Los Robles Open Space, Santa Monica Mountains, Ventura County, California (34.163226, −118.881964, elevation 370 m; Figs. 1, 2). The pond is oval with maximum size of 7 m by 5 m. No metamorphs were observed in the surrounding terrestrial habitat, but the presence of many tadpoles undergoing metamorphosis suggests that this late breeding event would result in metamorphs leaving the pond within a few days. Photographs of these tadpoles were submitted to the Reptiles and Amphibians of Southern California (RASCals) Citizen Science Project (iNaturalist 2365499) with additional photographs deposited in the Natural History Museum of Los Angeles County Photographic Collection (LACM PC 1998–2005).

Breeding was likely triggered by an unusually large rain event on the morning of 15 September 2015 that filled this previously dry, temporary pond. This rain event resulted in part from low-level moisture from the former Eastern Pacific Hurricane Linda. Weather data from Los Angeles indicate the storm produced the second wettest September day on record (6.07 cm). Data from the nearest weather station in the Santa Monica Mountains, which is at Deals Flat, ca. 11.5 km southwest of the breeding site, are available via climateanalyzer.org. At Deals Flat, 3.3 cm of rain fell in this unusual storm event, which is more rain than fell in the previous February (2.18 cm) or March (1.78 cm) when *B. boreas* typically breeds in this area. The rain event also took place in the fourth year of a severe drought in Southern California, during which time *B. boreas* breeding activity was greatly reduced.

To the best of our knowledge, the occurrence of tadpoles in November and an inferred breeding date in mid-September are the latest observations of breeding activity reported for *B. boreas*. Lemm (2006) noted that western toads breed from January to September, a slightly longer period than the January to July period suggested by Stebbins (2003), but no dates or locations of

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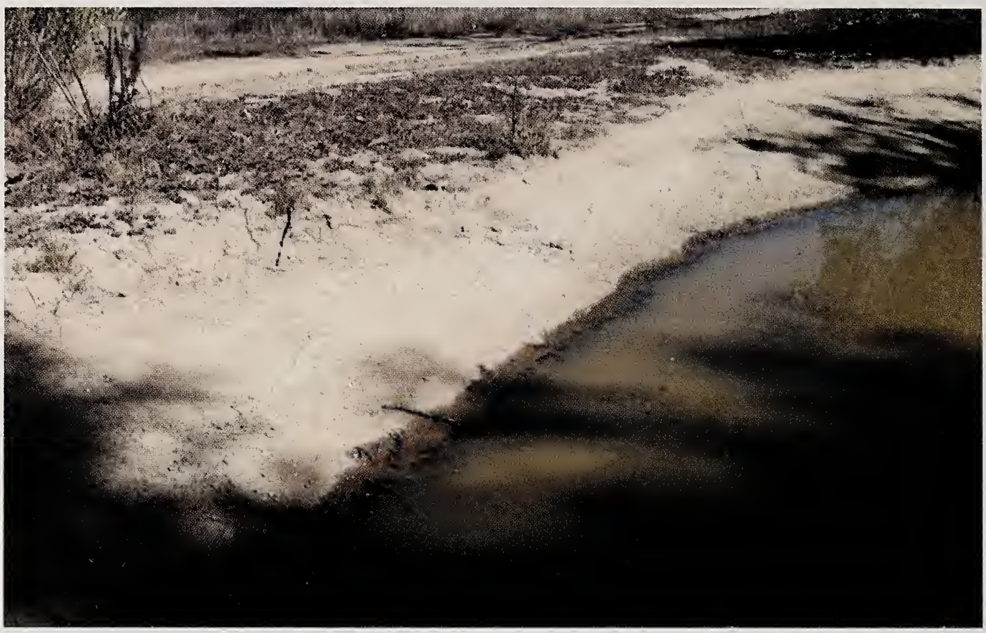

Fig. 1. Western toad (*Bufo boreas*) breeding pond observed 9 November 2015 in the Los Robles Open Space, Santa Monica Mountains, Ventura County, California. Note the ring of toad tadpoles lining the pond's edge (LACM-PC 2001).

September breeding activity were provided. Lemm (pers. comm.) could not reference specific observations of September breeding activity although he was certain he observed amplectant *B. boreas* in Southern California in September, with these individuals likely observed in the deserts. The latest specific observations of breeding activity are from August. Fetkavich and Livo (1998) reported late-season breeding in the southern Rocky Mountains with a clutch likely laid the first week of August 1997, though no tadpoles were suspected of surviving the winter. Sornborger (1979) also reported late-season breeding in *B. boreas,* this time at a high elevation site in the San Jacinto Mountains of Southern California. Here the primary breeding activity was in late April 1977, with subsequent breeding activity after a major storm event 15–17 August 1977 (19.3 cm of rain). Survivorship of these late-season tadpoles was not fully tracked, but mortality was suspected to be high due to the onset of cold weather.

A review of museum specimens also failed to find evidence of breeding as late as observed at the Ventura County pond. A search of the VertNet database for all *B. boreas* tadpoles returned 471 records (search conducted by requesting all *Bufo boreas* and *Anaxyrus boreas* records with the terms "tadpole OR tadpoles OR larva OR lot" and then eliminating any records that could not be confidently assigned as tadpoles). The latest collection month reported was September, for which there were seven lots (1.5% of total records): CAS 206431 and 206432 from 1 September 1998; CAS 209911 from 8 September 1999; UMMZ 151566 from 10 September 1962; CAS 180323 from 13 September 1991; UMMZ 151568 from 13 September 1967; and CAS 242852 from 16 September 2002. Photographs or specimens of these seven lots were examined to assess developmental stage following Limbaugh and Volpe (1957) and Gosner (1960). Based on developmental stage and collection date, the two latest season records are stage 24–25 tadpoles collected 13 September 1991 (CAS 180323) and stage 27–34 tadpoles collected 16 September

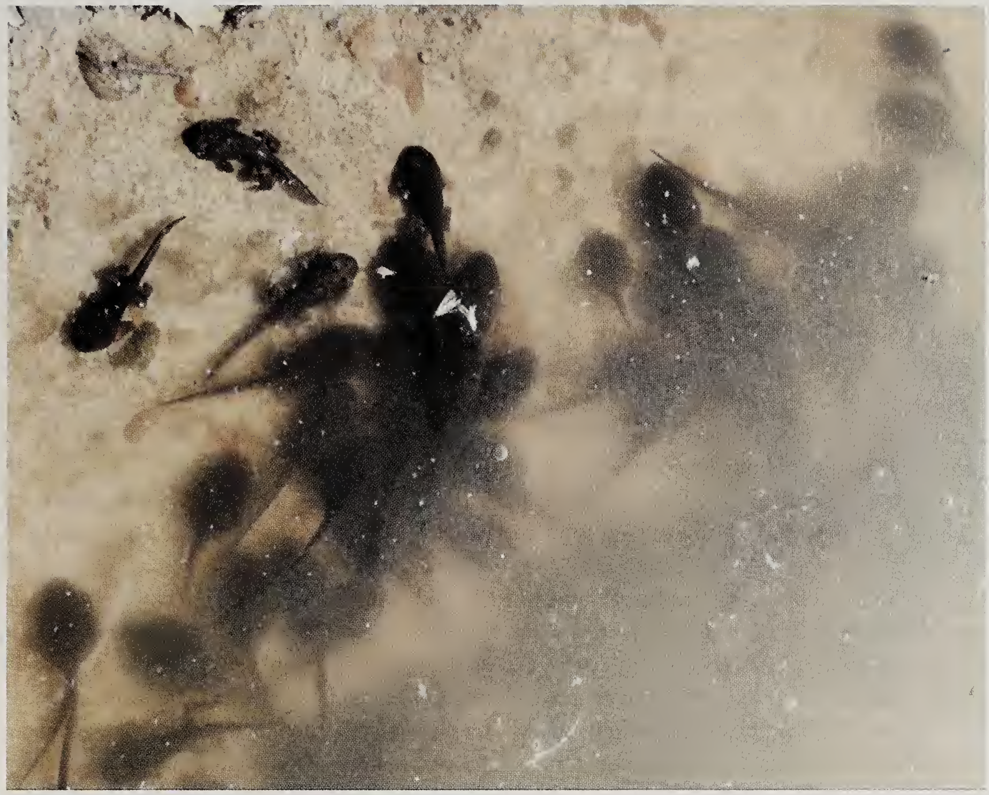

Fig. 2. Late-season western toad (*Bufo boreas*) tadpoles and early-stage metamorphs (up to Gosner Stage 43) observed 9 November 2015 in Ventura County, California (LACM-PC 2004 and iNaturalist 2365499).

2002 (CAS 242852). Both of these lots were collected in Southern California from relatively low elevation sites (1060 m and 775 m, respectively).

The Ventura County tadpoles were observed 9 November 2015, nearly two months later than available late-season tadpole records. Although this observation is much later in the season, it is possible that the Ventura County tadpoles and the two latest specimen records (CAS 180323 and CAS 242852) all result from breeding activity in September. Breeding dates for these records cannot be accurately estimated from developmental stage because development is strongly correlated with temperature and therefore will vary based on local conditions such as air temperature, percent shade, and pond vegetation and substrate. However, by generalizing based on developmental rates estimated by Limbaugh and Volpe (1957), it is possible that both CAS 180323 and 242852 resulted from early September breeding events. Breeding activity at the Ventura County pond was still later than that for these museum records because it likely occurred 15–17 September 2015, immediately after the large rain event.

Goldberg (2016) examined gonads of museum specimens collected between February and August from multiple localities in Riverside County, California, all more than 145 km east of the Ventura County pond and in much drier habitat with more variable summer rainfall. All adult males and females had mature gametes and could be reproductively active during this 7-month period. Although samples from September were not available, Goldberg suggested that the long activity period was consistent with a continuous pattern of reproduction in which individuals have prolonged periods of breeding readiness allowing them to take advantage of favorable conditions if they arise. This suggestion is consistent with our review of tadpole specimens as all seven lots collected in September likely resulted from late-season breeding activity, in which individuals bred long after the primary breeding period for that region.

This new record, in combination with our review of museum specimens and published reports (Sornborger 1979; Fetkavich and Livo, 1998; Lemm 2006; Goldberg, 2016), documents that *B. boreas* has a prolonged period of breeding readiness that can extend at least into mid-September. Thus, although most breeding activity occurs early in the active season, western toads are capable of breeding later in the year. At higher elevations, late-season breeding is unlikely to allow tadpoles adequate time to reach metamorphosis (e.g., Sornborger 1979; Fetkavich and Livo 1998), but at lower elevations in the southern portion of the range, late season breeding has a higher chance of tadpoles successfully completing metamorphosis, as likely occurred for the Ventura County tadpoles. The western spadefoot (*Spea hammondii*), which shares a similar distribution as *B. boreas* in Southern California, also has been found to have a more continuous reproductive mode allowing it to take advantage of rainfall events outside of the primary breeding period (Ervin et al. 2005; Ervin and Cass 2007).

In typical years in coastal Southern California, western toads breed during the late winter and spring rains. In spring 2015, however, rains were abnormally low due to drought conditions, and there was very little breeding activity in the region (based on long-term monitoring of sites in and around the Santa Monica Mountains National Recreation Area and by the low number of tadpole and metamorph observations submitted to the RASCals project). As a result, some adult toads likely had mature gametes and were capable of breeding following the atypical late-season rain event. Another possibility is that western toad females are capable of producing multiple clutches in a single breeding season, with breeding activity during the primary breeding season and a second clutch months later following storm events. We don't think this occurred here given the relatively dry spring and the low levels of breeding activity observed across Southern California. Among Nearctic *Bufo*, multiple clutches have only been documented in *Bufo cognatus* (Krupa, 1986). Nevertheless, future studies should investigate this possibility, particularly in areas with mild winters where late-season breeding is more likely to yield tadpoles that successfully reach metamorphosis.

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